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ENTAMOEBA PYRRHOGASTER N. SP., WITH NOTES ON OTHER INTESTINAL AMOEBAE FROM SALAMANDERS*

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The original purpose of the present investigation was the study of any *Entamoeba* that might be present in the intestines of the Japanese salamander, *Triturus pyrrhogaster,* and of the common red newt, *Triturus viridescens.* Such an amoeba, *Entamoeba pyrrhogaster* n. sp., was found in *Triturus pyrrhogaster* and grown in culture for six months. Observations on its morphology and life-history were made from living specimens and from preparations fixed in Schaudinn's fluid and stained with Heidenhain's haematoxylin.

During the study of the fecal material of these two species of salamanders, two other amoebae were incidentally recovered, cultured, and studied in fresh and stained preparations. These included *Vahlkampfia salamandrae* n. sp., from *Triturus pyrrhogaster,* and, from *Triturus viridescens, Copramoeba salamandrae* herein described as a new genus.

So far as can be discovered no intestinal amoebae have been studied from salamanders. Although Chatton (1910) reported an amoeba from *Triton palmatus,* and Alexeieff (1912a) found a similar one in *Triton taeniatus,* neither author fully described the amoebae seen. Wenyon (1926) suggested that they were probably *Entamoeba ranarum.* Pearse (1932), in a report of parasites of Japanese salamanders listed an amoeba, not named, in *Pseudosalamandra stejnegeri.* Although *Triturus pyrrhogaster* was studied, no parasitic amoebae were seen in it. Rankin (1937) reported *Entamoeba ranarum* from *Triturus viridescens,* although he gave no evidence to indicate that the amoeba found was identical with that of the frog.

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MATERIALS AND METHODS

Source of Material

A constant source of material was at hand in the Japanese salamanders, *Triturus pyrrhogaster,* which had been ordered from the Nippon Goldfish Company of California for use in other experimental work at the laboratory, and in the common red-spotted newt, *Triturus viridescens,* collected from local ponds in October. These two species of salamanders were kept in separate tanks and occasionally fed pieces of guinea-pig liver. While in the laboratory they never came in contact with frogs, although the latter had been present in the ponds with *Triturus viridescens.*

Salamanders to be studied were killed by decapitation, and pieces of their recta were removed to a 0.5 per cent physiological salt solution on glass slides for microscopic examination of the fecal material. Samples of this material were also transferred to test tubes of media for cultivation at room temperature.

Culture Media

Four culture media were tried and proved successful for the three amoebae that were found. These included the following:

1. Tanabe and Chiba's (1928) medium, in which the authors grew *Entamoeba coli* and *Entamoeba histolytica* from man, and in which Sanders (1931) grew *Entamoeba ranarum.* Slants were made with agar and asparagin and covered with 1:8 rabbit serum—Ringer's. A loopful of rice starch was added to each tube of this and the following media.

2. Ratcliffe and Geiman's (1938) medium developed for culture of *Entamoeba invadens* from reptiles. This was a liquid medium containing gastric mucin (Difco) and "ground alum" salt in distilled water.

3. Cleveland and Collier's (1930) medium (modified), used for *Entamoeba histolytica* and by Sanders (1930, '31) for *Entamoeba terrapinae* and *Entamoeba ranarum.* Slants were made with liver infusion agar (Difco) and covered with 1:6 rabbit serum-saline. Rabbit serum was used in this and in the following medium, instead of horse serum as originally called for, because horses were not available.

4. Sanders' (1930) medium (modified), used for *Entamoeba terrapinae,* consisting of 1:95 rabbit serum-saline.

Cultivation

To initiate cultures, tubes of the four media were inoculated with pieces of fecal material in which amoebae had been observed, then incubated at room temperature for a week. Samples from each tube were examined microscopically, and transfers to fresh media were made by adding a few drops of liquid and debris from the cultures which were

most flourishing. At first weekly subcultures were made, but it was found that, in most cases, the cultures required more than a week for good growth, reaching a maximum after four or five weeks, so transfers were made less often. Some cultures remained good for as long as three months.

Amoebae encysted freely in all the cultures, but a special effort had to be made to find the excystation stages. Washed cysts were stored for eight days in the refrigerator, before being transferred to fresh media at room temperature. When no excysting forms were observed at the end of seven hours, they were returned to the refrigerator for three more days, then left at room temperature. At the end of the day there had been no excystation, but six days later, when they were next observed, some metacystic forms were found and slides were made. A culture from these cysts was established. On a second occasion washed cysts were stored in the refrigerator for 32 days, then kept at room temperature. In this case metacystic amoebae were not observed in fresh preparations, but on stained slides made after seven hours a few were present. Although the cultures were kept for a month, the metacystic amoebae did not grow well. Finally washed cysts were not put in the refrigerator, but kept at room temperature, and a few active amoebae were observed after 12 days.

Enflagellation

Determination of the genera of amoebae other than *Entamoeba* is dependent, in some instances, on the production of flagella by the amoeba. Attempts to bring this about were made by ringing a slide with vaseline, placing on it a drop of amoebae in culture fluid, and flooding them with cold tap or distilled water. When a coverslip was placed over them, the amoebae, thus sealed in, could be observed for several days. Amoebae were also grown on plain and asparagin agar plates, then flooded with distilled and tap water, and observed at intervals under the microscope.

Cross Infection

To determine the infectivity of the *Entamoeba* of the salamander for frogs, an attempt was made at cross infection. Three frogs, *Rana pipiens,* were separated from a group of frogs which had been isolated and without food for about six months. Of the three, one was pithed, and its intestinal contents microscopically examined, then placed in the media prepared for the *Entamoeba* from the salamander, but also known to be good for *Entamoeba ranarum.* The other two were washed and placed in a covered dish of shallow water at room temperature. For three days they were fed on guinea-pig liver.

The two surviving frogs on the third day were each forcibly fed a pipette full of culture containing trophozoites and cysts of the *Enta-*

moeba of the salamander. They were then washed in running water and placed in a clean dish with fresh tap water. Water and fecal material from the old dish were examined and placed in culture.

The frogs were fed guinea-pig liver for three more days. Daily they were washed and changed to fresh water. Samples of the water in which they had been kept were examined microscopically and placed in culture media. After the fourth day subsequent to the inoculation with amoebae, feeding was discontinued. The water was, however, changed and examined, and samples of it were placed in culture media on four more occasions within the next two weeks.

On the 19th day after the inoculation with amoebae, one of the experimental frogs was pithed and its intestinal contents were examined and put in culture media. The third frog was kept for another week without food before it too was killed for examination and culture of the intestinal contents.

Stained Slides

Permanent preparations were fixed in Schaudinn's fluid with glacial acetic acid at room temperature, and stained with Heidenhain's haematoxylin. A few slides were fixed in the same way and stained by Feulgen's method to show up the chromatin material.

RESULTS

The experimental results of the present work may be divided into three parts, relating to the three amoebae from salamanders which have been cultured, namely, *Entamoeba pyrrhogaster* n. sp., *Vahlkampfia salamandrae* n. sp. and *Copramoeba salamandrae* n. g. et sp.

1. *Entamoeba pyrrhogaster*

Culture

Successful cultures were started of three strains of *Entamoeba* isolated from intestinal contents of three salamanders (*Triturus pyrrhogaster*). One strain after six months was still in good condition. Amoebae grew equally well in the four different media. In general, culture tubes showed a maximum growth after the third or fourth week from the time of inoculation. About a month after the original cultures were started, cysts were abundant enough to be readily observed, and the number of trophozoites decreased. In some cultures a second peak of growth was observed after a month or two.

Trophozoite

Trophozoites are easily visible under the microscope as clear bluish bodies of irregular shapes, containing shiny refractive starch grains. Typically, movement is accomplished by the formation of large explo-

sive pseudopodia, often as wide as the whole body of the amoeba. The pseudopodia, at first of clear hyaline ectoplasm, soon become filled with the tumbled granules of the endoplasm, as the bulk of the amoeba flows in, and the animal progresses, changing direction often by sending out new pseudopodia. At other times the amoeba may send out one or more fine filamentous pseudopodia of clear ectoplasm only; these have been seen to attain a length several times that of the body proper.

Camera lucida drawings of many amoebae from stained slides show that the sizes of most of them range from 7 to 40 μ (Figs. 1, 3, 5), although there were a few larger ones. The largest observed on a stained slide (Fig. 2) measured 90 by 70 μ, but a few huge living ones were as large as 150 by 90 μ. These last, seen only on four occasions, were typical in all other respects.

The nucleus was seldom seen in living amoebae, but could be easily observed in stained preparations, its size, varying with the size of the amoeba, from 2 to 18 μ. Typically, the nucleus has a fine peripheral rim of chromatin granules and a small central karyosome, often eccentric in position (Figs. 1, 7). The larger nuclei show the outer portion as a deeply staining region containing chromatin granules of various sizes, and the inner portion as a lighter area with from one to three dark granules (Figs. 2, 5).

Stained preparations show that the chief article of diet of the culture amoebae consists of rice starch grains, with which the cytoplasm may be quite thoroughly packed. Usually no bacteria have been ingested. A number of cases of cannibalism were observed, an example being shown in Fig. 5, where a single large trophozoite has eaten four cysts of *Entamoeba*.

The cytoplasm, as seen in stained preparations, appears somewhat alveolar or granular. The ectoplasm can be distinguished from the endoplasm, only where pseudopodia have been actively forming. Then it appears paler and more homogeneous (Figs. 3, 30, 31). In degenerate forms from unhealthy cultures the cytoplasm may be vacuolated, but at no time is a contractile vacuole present.

A few cases of nuclear division were observed on stained slides. In some amoebae, as in Fig. 11, chromatin material is drawn out as an indistinct mass in the center of the elongating nucleus. In one large amoeba, where division could be seen more clearly, there were present two pairs of deeply staining granules connected by wavy threads (Fig. 4). Similar figures were observed in two cyst nuclei (Figs. 13, 24), although less clearly. These granules may be chromosomes derived from part of the peripheral chromatin, the remainder of which stays on the nuclear membrane. Some amoebae with two nuclei were found (Fig. 8), indicating that cytoplasmic division occurs after nuclear division is completed (Fig. 9).

Cysts

In cultures where cysts were newly formed, small amoebae containing little or no starch were found (Fig. 10). These were probably precystic forms, having used up most of their food material, and decreased in size by repeated division.

Cysts, which develop in great numbers, appear very refractive and bluish in fresh preparations, and are smaller than the trophozoites. Stained preparations demonstrate their thin walls, often with bacteria or debris adhering to the outside (Fig. 25). Measurements of camera lucida drawings of a number of representative cysts show that they range in size from 5 to 15 μ; however, most were under 10 μ. Immature cysts typically have a large glycogen vacuole and several deeply staining chromatoidal bodies in the shape of small rods or irregular masses (Figs. 11–27). These represent a reserve food supply and disappear as the cysts reach maturity (Fig. 28). Within the cyst two nuclear divisions usually occur, producing four cystic nuclei (Figs. 11–23); but a few larger cysts were observed with as many as 16 nuclei (Figs. 25–27), possibly formed from amoebae that had been binucleate at the time of encystation.

Metacystic Amoebae

Metacystic amoebae emerge as quadrinucleate forms (Fig. 29) which promptly ingest many bacteria, becoming filled with chains of bacilli. Few ate rice starch, although they were grown in media similar to that in which trophozoites had ingested the starch but no bacteria. As the cytoplasm of the amoebae increases the nuclei also grow larger (Figs. 30, 31) and divide.

The metacystic amoebae exhibit variable nuclear conditions. Let N represent the cystic nucleus, and n, the smaller metacystic nucleus, the product of division of N. Various combinations of N and n have been observed, but in no amoebae were more than a total of five nuclei found, indicating that cytoplasmic division follows closely upon nuclear division. The following combinations were found (Figs. 29–46): NNNN, NNNn, NNN, NNnnn, NNnn, NNn, Nnnn, Nnn, nnnn, nnn, nn, and n.

Cross Infection

In an attempt to determine the host specificity of the *Entamoeba* of the salamander, two frogs were fed the trophozoites and cysts of the *Entamoeba,* as described above under materials and methods. It is believed that these frogs were not previously infected with *Entamoeba ranarum,* because examination and subsequent culture of their fecal material for three weeks, in media known to be favorable for *Entamoeba ranarum,* failed to show the presence of any cysts or amoebae. Exami-

nation of the intestinal contents of two other frogs from the same original lot confirmed the absence of *Entamoeba ranarum.* The only protozoa found in the frogs that were killed, and in the water and fecal material of the experimental animals before feeding with the parasite of the salamander, were *Trichomonas, Tetramitus,* other flagellates not identified, *Opalina,* and *Colpoda.* A few *Entamoeba* cysts were observed in the feces a day after the cysts had been ingested by the frogs, but not subsequently.

When cultures of contents of the intestines of the killed frogs, and of the feces and water of the experimental animals, were examined three weeks after the frogs were fed amoebae, *Entamoeba* was found in only one case. This was from feces collected the day after ingestion of the cysts, and inoculated into media for culture.

When the experimental animals were killed, one 19 days and the other 26 days after they had been fed amoebae, no amoebae or cysts were found in their intestines, nor did any grow from the intestinal contents placed in culture media. However, there was a heavy infection with *Trichomonas* and *Tetramitus.*

Thus the evidence indicates that the frogs had not been infected with *Entamoeba ranarum. Entamoeba* from salamanders were ingested, and cysts passed through the intestines, but, since the amoebae were not viable in the intestine of the frog, the infection did not become established.

2. *Vahlkampfia salamandrae*

Culture

A second amoeba, isolated from *Triturus pyrrhogaster,* has been grown in culture for five months. It is smaller than the *Entamoeba,* measuring about 17 μ on stained slides, and is easily distinguishable from the latter in both fresh and stained preparations by the appearance of cytoplasm and nucleus. Study indicates that it is a species of *Vahlkampfia.*

This amoeba grows best in Geiman's and in Tanabe's media, and least well in Cleveland and Collier's, in which bacteria multiply too rapidly. A good thick culture may be obtained within a week after subculture, and may last as long as five weeks without diminishing. The amoeba was also grown on asparagin agar plates.

Trophozoite

The living amoeba is fairly active, sending out numerous clear hyaline pseudopodia in all directions. The cytoplasm is filled with bacteria, which appear as refractive granules, often in vacuoles. The nucleus shows as a greenish blue refractive sphere, surrounded by a clear area. Amoebae grown on agar plates assume, in general, a more elongated

and flattened appearance than those grown in culture tubes. They also are more vacuolar and contain contractile vacuoles not present in forms from tube cultures. These empty once or twice a minute.

The most striking structure in the stained amoeba is a large deeply staining karyosome, 2 or 3 μ in diameter, either homogeneous in appearance or somewhat vacuolar, similar to the macronucleus of many ciliates. Surrounding the karyosome there is a clear region separating it from the nuclear membrane. Along the latter, in most resting nuclei, there are arranged either a few coarse, or many fine chromatin granules (Figs. 47–49). In some amoebae the outer nuclear membrane is invisible (Figs. 50, 53).

Division

A number of division stages, typical for the genus *Vahlkampfia,* have been observed. The first indication in the nucleus of approaching division is an accumulation of chromatin granules around a dense karyosome (Fig. 52). The karyosome elongates while the chromatin granules stay concentrated in the central region (Figs. 54–56). In the metaphase picture (Figs. 57, 58) the karyosome is divided into two polar caps between which chromatin granules appear to be on an equatorial plate. The granules, probably chromosomes, are too fine to be counted. In the anaphase the granules have evidently divided and one set passes to each pole (Fig. 59). Late anaphase stages were not observed, but binucleate amoebae (Fig. 61) were found, indicating that nuclear division is completed before cytoplasmic division begins (Fig. 62). One quadrinucleate amoeba was seen dividing into two binucleate ones (Fig. 63). In this case nuclear division was evidently more rapid than cytoplasmic division.

Enflagellation

Several attempts were made to produce flagellated forms by flooding the amoebae on plates and slides with tap and distilled water. In most instances these proved unsuccessful, but twice, when very cold water was used, a few flagellated forms were discovered. However, the culture as a whole never enflagellated. The flagellated amoeba has an elongated ovoid shape and moves actively through the water. A contractile vacuole is usually present in the posterior end. Stained slides show the presence of two flagella arising from a single basal body which is connected by a fine thread to a granule, probably a centriole, at the periphery of the nucleus (Figs. 71, 72). Few stages of enflagellation were found, but in one stained amoeba (Fig. 70) the karyosome appears to be giving rise to a centriole from which the basal body and the flagella will arise.

Cysts

Cysts of a fairly uniform size, about 13 μ in diameter, are formed

readily in culture, smooth and brownish in fresh preparations. On stained slides three or more pores may be seen. In normal cysts the walls inside and out are smoothly spherical, but quite thick and impenetrable to fixative and stain. As a result, on stained slides, they often appear collapsed and irregular, like crenated red blood cells. Cysts are often found in large clusters of 20, 30 or more.

The impenetrability of the cyst walls makes observations of the enclosed amoebae difficult. In many no nucleus can be discerned (Fig. 66); in some it appears as a pale sphere with a slightly darker central mass (Figs. 67, 68); in no case was it as deeply stained as in the trophozoite. No cysts were found with more than one nucleus.

Excystation

Although no excysting amoebae were found on stained slides, several were observed under oil immersion in the living state. Plate VI shows camera lucida drawings of an emerging amoeba. The beginning of excystation was not observed. When first seen, the amoeba was half out of its cyst, with the nucleus inside, but 10 minutes later it had retreated almost wholly within the cyst again. It took 20 minutes more before the amoeba had emerged, except for a small knob of cytoplasm. At this stage the amoeba was lost. It is estimated that the process of excystation takes at least one hour, and possibly longer.

Although more than one pore is present in each cyst (Fig. 69), the amoeba emerged through only one in cases observed. That the nucleus does not divide in the cyst is an indication that the encystment is for protection rather than for reproduction as part of the life-history of the amoeba.

3. *Copramoeba salamandrae*

Culture

The third species of amoeba was recovered from the red-spotted newt, *Triturus viridescens*. Two strains were isolated from the intestines of two different salamanders; of these one culture was still flourishing after six months. Although this amoeba grew well in all media, cultures in Tanabe and Chiba's medium were thickest. It also grew rapidly and extensively on plain or asparagin agar plates.

Trophozoite

The living amoeba looks much like the *Vahlkampfia,* although it is a little smaller, 4 to 14 μ on stained slides, and less active. The hyaline ectoplasm can be distinguished from a granular endoplasm only at times, when explosive ectoplasmic pseudopodia are thrown out. Usually the amoeba moves ahead by a flowing of the whole body, as in *Amoeba proteus*. It feeds on both yeast and bacteria that are present in the media.

A contractile vacuole is plainly visible in amoebae grown on both liquid and agar media, contracting much less frequently than does that of the *Vahlkampfia* on agar, only once in five minutes. The karyosome of the nucleus in the living amoeba appears as a smaller, brighter, and more distinctly refractive body than does that of the *Vahlkampfia.* It is surrounded by a clear halo.

In stained preparations the nucleus typically consists of a deeply staining, homogeneous spherical karyosome, smaller than that of *Vahlkampfia,* and more like the micronucleus of a ciliate, surrounded by a clear area which separates it from the nuclear membrane (Fig. 73). Often fine chromatin granules are visible on the inner edge of the nuclear membrane, while delicate linin threads connect the latter with the karyosome. At times the outer granules and connecting threads are invisible, and the nucleus appears only as a deeply staining karyosome surrounded by a clear area (Fig. 76). Sometimes only the central part of the karyosome stains deeply, while the outer part is a dark grey color (Figs. 74, 75). In a few the deeply staining part of the karyosome has the appearance of a curved line (Fig. 78), a circle (Fig. 79), or an irregular dot (Fig. 80), but no spindle, as described for the dividing nucleus of *Hartmanella,* or polar caps with equatorial plate, as found in *Vahlkampfia,* were found, although many amoebae were studied.

Division has not been satisfactorily worked out, but, as far as has been found, the process is as follows: The karyosome loses some of its affinity for stain, so that only the central part is deep black (Fig. 75). This center elongates and divides amitotically (Figs. 81–83), resulting in a karyosome with two dark centers (Figs. 84, 85). In these stages the outer membrane is usually not seen. The whole karyosome then divides into two, equal or unequal, parts (Figs. 86–88). The outer membrane also divides and the normal trophozoite nucleus reappears (Fig. 89). Two cases were observed with two dividing nuclei present in a single amoeba (Fig. 90).

A number of amoebae were observed in which no nucleus could be found, although others on the same slide had deeply staining nuclei. At first it was thought that division with the formation of a spindle might be taking place, but careful study gave no evidence of this. The nucleus may have been extruded by the amoeba; it may have broken out during fixation and staining; or it may have been in a degenerate condition that prevented staining. In one amoeba (Fig. 93) it appeared that the nucleus was being extruded.

Cysts

The cyst in fresh preparations readily distinguishes this amoeba from *Vahlkampfia.* It appears to have a thicker wall, with bacteria and debris often adhering to the outside. Cysts may occur in masses of 100

or more. The amoeba inside looks greenish and granular, usually with one or more clear refractive bodies in it.

Stained cysts vary in size from 5 to 10 μ inner diameter. The inner wall is usually smoothly spherical, but the outer one is often irregular and wrinkled (Fig. 94). The amoeba usually lies free from the cyst wall. There may be one large deeply staining nucleus (or karyosome?), more than one, or none at all (Figs. 94–108). The staining reaction of the nucleus seems to be quite variable. Apparently after it has divided into several nuclei, these lose their affinity for the iron haematoxylin stain and appear greyish (Figs. 106–108).

Different combinations of large and small nuclei have been found, but in any cyst there are not more than two sizes of nuclei. Letting N represent large nuclei, and n, small ones, the following combinations have been found: N, NN, Nnn, nnn, Nnnn, Nnnnn, nnnnnn, Nnnnnnn, Nnnnnn. The last three cases include some light and some dark nuclei. Most frequently observed was the case of Nnnn. Since there seemed to be little order to the nuclear combinations, it was at first doubted that the bodies were nuclei. However, slides stained by Feulgen's technique, which is specific for chromatin, showed the presence of lavender bodies corresponding to the black ones of iron haematoxylin staining, so they must be of a chromatin nature.

Excystation

It would seem that the amoeba divides in the cyst and the individuals emerge in a uninucleate condition, since such odd combinations of cyst nuclei are encountered, and since no trophozoites with more than two nuclei have been observed. Fig. 108 of a stained cyst suggests the formation of amoebulae in the cyst. Fig. 109 may similarly show a group of such amoebulae which have just emerged. In a plate culture flooded with Geiman's fluid after all the original amoebae had been encysted for over a week, some small uninucleate amoebae, 4 μ in diameter were observed. These had probably recently excysted.

Enflagellation

Attempts were made to produce flagella, as with *Vahlkampfia,* but at no time were they successful. The addition of tap or distilled water to a plate culture caused no change in the amoebae.

DISCUSSION

I. *Entamoebae*

In all groups of animals the few cross-infection experiments that have been made show that with *Entamoeba,* as with other protozoan parasites, there is a high degree of host specificity. It has thus become the

custom to give an *Entamoeba* from a new host a new species name, if for no other reason than that the host is an addition to the list of known hosts of *Entamoeba.* Characters of the amoebae on which species are separated include: size range of trophozoites and cysts, character of the cyst, number of cyst nuclei, appearance of the chromatoidal bodies, and nuclear structure.

The difference in host would customarily be enough to separate the *Entamoeba* found in the salamander, *Triturus pyrrhogaster,* from the species found in animals other than amphibia. There might, however, be some question as to its affinity with *Entamoeba ranarum.* Rankin identified amoebae recovered from North Carolina *Triturus viridescens* as *Entamoeba ranarum,* considering them accidental parasites, since frogs in the same locality were also infected with *Entamoeba.* In this case, although no frogs had been present with the salamanders for some weeks prior to the study of their intestinal contents, they may have come in contact with *Entamoeba ranarum* some time before. If so, the amoebae, having lived in them, could be considered as much a parasite of the salamander as of the frog, and not merely accidentally present. However, the fact of host-specificity, as proved by experiments with various amoebae and other protozoan parasites, would indicate that the *Entamoeba* found in frogs and in salamanders should be placed in different species.

The trophozoite of *Entamoeba ranarum* has been reported by Dobell (1909), Sanders (1931), Mercier and Mathis (1918), and Taliaferro and Fisher (1926) as measuring between 10.4 and 40 μ. The amoeba described from the salamander varied for the most part from 7 to 40 μ, with a few as large as 150 μ; thus its size is approximately that of *Entamoeba ranarum,* except for the extremes. The cyst size of the two amoebae is likewise similar, 6 to 20 μ for *Entamoeba ranarum,* and 5 to 15 μ for the *Entamoeba* of the salamander.

As regards the character of the cyst of *Entamoeba ranarum,* Dobell reported that it was surrounded by a delicate membrane and contained a glycogen vacuole and masses of chromatoidal bodies; each nucleus had a mass of chromatin in the center, as a "heap of loosely packed granules"; there were four cyst nuclei. Mercier and Mathis recognized two kinds of cysts, "gamogonique" with four nuclei, and "schizogonique" with as many as 20; chromatoidal bodies were found only in the "gamogonique" cysts. Sanders described the cyst wall as jelly-like and irregular; she figured rod-like and irregular chromatoidal bodies, and four cyst nuclei. The *Entamoeba* from the salamander has a thin walled cyst, somewhat irregular on its outer surface, but not sticky or gelatinous in appearance. In fact, it is often hardly visible. As in the amoeba from the frog, there are rod-shaped and irregular chromatoidal bodies, and

typically four nuclei. The karyosome of the cyst nucleus is finer than that described by Dobell, but similar to that figured by Sanders. Multinucleate cysts may be similar to those called "schizogonique" in *Entamoeba ranarum* by Mercier and Mathis, but no evidence of gamogony was found.

Finally, although the experimental attempt to infect *Rana pipiens* with the *Entamoeba* of the salamander, as carried on here, was too limited to be decisive, the negative results obtained do indicate that this *Entamoeba* differs from *Entamoeba ranarum,* since it is apparently not infective to frogs. The results support the hypothesis of host-specificity of *Entamoeba* spp.

Hence, it is here proposed, that the *Entamoeba* found in the large intestine of *Triturus pyrrhogaster* be recognized as a species distinct from *Entamoeba ranarum* of frogs, and be given the name *Entamoeba pyrrhogaster,* n. sp.

II. *Coprozoic Amoebae*

General

There is some confusion in the literature regarding the definition of genera of coprozoic amoebae, especially of *Naegleria, Vahlkampfia,* and *Dimastigamoeba.* Besides these, Alexeieff (1912a) proposed the genus *Hartmannia* for a coprozoic amoeba with no polar caps, but with centrioles or no structures at the poles of a spindle in division, and with most, or all, of the chromatin material on the equatorial plate. In a later paper of the same year (1912b) he recognized Chatton's criticism that the name *Hartmannia* was already in use as a generic name for a pulmonate, and revised his genus to *Hartmanella,* which stands today, along with his definition of the genus.

Blochmann (1894) separated Kleb's *Dimorpha radiata* from the genus *Dimorpha,* giving it the generic name *Dimastigamoeba.* His reason was that *Dimorpha radiata* did not have the heliozoan type of pseudopodia that characterized *Dimorpha,* although they both had flagella. Examination of Kleb's (1892) figures of *Dimorpha radiata* shows that the amoeba possesses one flagellum directed forward and one backward. Sometimes it is ovoid and elongate, broader at either the posterior or the anterior end. Sometimes it sends out lobose or filiform pseudopodia. Division in the flagellate stage is figured. There is no indication of an entirely amoeboid phase.

Alexeieff (1912a) proposed the genus *Naegleria* for a limax amoeba characterized as follows: lobose pseudopodia, contractile vacuole, large karyosome and little peripheral chromatin, flagellate stage with two flagella, uninucleate cysts with chromatoidal bodies. Chatton (1912) challenged the priority of Alexeieff's name, claiming that he had published, a month earlier, a description of the genus under the name *Vahl-*

kampfia. He said that *Naegleria* was thus a synonym of his *Vahlkampfia,* although he reported no flagella formation in the amoeba he studied. He upheld the name *Trimastigamoeba* Whitmore for an amoeba that may have three flagella, and *Dimastigamoeba* Blochmann for one which may have two flagella, one trailing, and with multiplication in cysts.

In reply to Chatton's criticism, Alexeieff (1912b) accepted the genus *Vahlkampfia* Chatton and Lalung-Bonnaire, relegating to the position of synonyms *Dimastigamoeba* Blochmann, *Trimastigamoeba* Whitmore, and *Naegleria* Alexeieff. He believed that, in *Trimastigamoeba,* Whitmore saw three flagella where there were only two. He was convinced that *Dimastigamoeba* and *Vahlkampfia* were the same, but did not commit himself on which name should be retained.

Calkins (1913) recognized *Vahlkampfia* as a generic name for limax amoebae in which no flagellate stage has been found, and left *Naegleria* to those with a biflagellate stage and a contractile vacuole. Wilson (1916) in connection with the distinction between *Naegleria* and *Dimastigamoeba* says:

"Alexeieff says there is a complete resemblance between *Vahlkampfia punctata* and *Dimastigamoeba radiata* Blochmann (1895). If this resemblance proves valid, then *Dimastigamoeba* will have to be used instead of *Naegleria* for biflagellated amoebas.

"Should *Dimastigamoeba* be used for the generic name of the biflagellate amoebas, they would be placed in a genus in which the other species are flagellated throughout their free state in so far as is now known, while in these forms a flagellate condition is occasional and temporary. . . . The genus was created for two amoeboid flagellates, and unless these have a non-flagellate amoeba stage, *Naegleria* should be retained as a generic name for the biflagellated amoebas, which seem more closely related to the *Rhizopoda* than to the *Mastigophora.*"

Wenyon (1926) in reference to *Vahlkampfia* says: "Chatton and Lalung-Bonnaire (1912) created the genus *Vahlkampfia* for amoebae showing this type of nuclear division (i.e. with polar caps) and possessing pores in the cyst wall. Flagellate forms of the amoebae were not observed, but the conditions necessary for the production of flagellate forms were not provided by them. Several other observers have described amoebae which show nuclear division of the same type, and which have not been noted to give rise to flagellate forms. It does not seem improbable that most, if not all, of these forms would produce flagellate stages if the necessary conditions existed. *Dimastigamoeba gruberi* remains as an amoeba on agar plates, or in cultures in egg-albumen water and other media, and does not become a flagellate unless a sudden change occurs in the medium, as, for instance, that produced by

the addition of tap water. If this is done, flagellates appear in three or four hours, but they revert to the amoeboid form again in about a day. It is probable that many of the amoebae which have been placed in the genus *Vahlkampfia* would be capable of transformation into flagellate forms if they were similarly treated. It is possible, however, that some of them would not. . . . As Chatton and Lalung-Bonnaire actually observed markings which were undoubtedly pores on the cyst wall, it is probable they were dealing with an organism belonging to the same genus (i.e. *Dimastigamoeba*). In this case both the names *Naegleria* and *Vahlkampfia* are really synonyms of *Dimastigamoeba.*" Wenyon thus dispenses with the genus *Naegleria,* placing most of its amoebae with the *Dimastigamoeba,* and leaves *Vahlkampfia* for amoebae of the same type which do not form flagella.

Doflein and Reichenow (1929) follow Calkins' classification in putting *Vahlkampfia*-like amoebae possessing a flagellate stage and having a contractile vacuole and uninucleate cysts, under the genus *Naegleria.* They place *Dimastigamoeba* with *Cercomonas* Dujardin 1841, a genus of protozoa normally flagellated but feeding in an amoeboid manner.

Kudo (1931) characterizes *Naegleria* Alexeieff (= *Vahlkampfia* Chatton and Lalung-Bonnaire in part) as a small ameoba with two flagella at times, with lobose pseudopodia, distinct ectoplasm and endoplasm, a large endosome in the nucleus, contractile vacuole, uninucleate cyst, and a bacterial feeder. *Trimastigamoeba,* according to him, has three flagella, and a uninucleate cyst with smooth walls. *Vahlkampfia* has a large endosome and peripheral chromatin in the nucleus which divides by the formation of polar caps, and a small uninucleate cyst with perforated walls. In the latest edition of his Protozoology Kudo (1939) follows Wenyon in placing *Naegleria* under *Dimastigamoeba,* as diphasic amoeba, and leaving *Vahlkampfia* for monophasic forms without a flagellate stage.

In summary the present status of the above genera, as agreed upon by most authors, is:

1. That *Dimastigamoeba* be reserved for amoeboid flagellates;

2. That *Vahlkampfia* include all limax-like amoebae dividing by the formation of polar caps, without a flagellate stage, and with or without contractile vacuoles;

3. That *Naegleria* include *Vahlkampfia*-like amoebae with a biflagellate stage, and with contractile vacuoles.

The present study of a *Vahlkampfia*-like amoeba obtained from fresh fecal material of *Triturus pyrrhogaster* seems to throw some light on the subject. According to the above summary it should be placed in the genus *Naegleria,* since it is usually amoeboid but occasionally forms

flagella. However, it seems more fitting to place it in the genus *Vahlkampfia* for the following reasons:

1. During most of its culture history it behaves like a typical *Vahlkampfia* without a contractile vacuole, and without flagella.

2. When grown on agar plates, it acquires a contractile vacuole, a change similar to that undergone by salt water protozoa when transferred to fresh water.

3. Most of the ordinary methods of producing flagella failed. Wenyon (1926) says that *Dimastigamoeba,* when grown on agar plates then flooded with tap water, soon enflagellates; likewise, Wilson reported that "to turn a whole film of amoebas into flagellates it is only necessary to add a few drops of distilled water to the cover-slip containing them." She also found flagellates occurring regularly in cultures, although few in number in comparison with the amoeboid forms.

4. In the few cases where flagellated forms did appear, a number of hours after the addition of very cold tap water, never did the whole culture so change, but only a few amoebae, and these were not disturbed by mechanical agitation of the medium, as Miss Wilson described for her *Naegleria gruberi.*

Wenyon thought that all *Vahlkampfia,* if given the right conditions, might produce flagella. This case seems to fall in line with his idea. If the study had been stopped after numerous attempts to produce flagella, but before the successful one, there would have been little doubt that the amoeba was a species of *Vahlkampfia.* Perhaps the case of *Vahlkampfia patuxent* from the oyster, studied by Hogue (1921) is similar, for she failed to obtain flagella by all the ordinary methods which are used for *Dimastigamoeba gruberi.*

It is therefore proposed that the term *Naegleria* be dropped, and that all amoebae of the limax-type dividing by the formation of polar caps, with or without contractile vacuoles, with or without demonstration of their development of flagella at some time in their culture history, provided that they spend most of their life-history in the amoeboid stage, be placed in the genus *Vahlkampfia* Chatton and Lalung-Bonnaire 1912. *Dimastigamoeba* Blochmann 1894 is then left for those protozoa which, though also dividing by the formation of polar caps, are usually biflagellate, feeding as amoebae and sending out pseudopodia at times.

Vahlkampfia

Many species of *Vahlkampfia,* both free-living and parasitic have been named. Most of these differ from each other in details of nuclear division, or only in habitat. As with *Entamoeba* it is customary to name *Vahlkampfia* from different hosts as different species, even though they appear very similar. Thus *Vahlkampfia froschi* (Hartmann) is found

in frogs; *Vahlkampfia lacertae* (Hartmann), in lizards; *Vahlkampfia mucicola* (Chatton), on the gills of marine fish; *Vahlkampfia patuxent* Hogue, in oysters; and *Vahlkampfia albida* (Nägler), *Vahlkampfia tachypodia* (Gläser), *Vahlkampfia diplogena* (Bělař), and others are free-living.

Without any definite attempt to prove that the amoeba found in *Triturus pyrrhogaster* differs from all the other *Vahlkampfia* described, beyond stating that it comes from a different host, it is proposed that it be called *Vahlkampfia salamandrae,* n. sp.

Copramoeba

This amoeba fits into no genus of amoebae so far described. The presence of a contractile vacuole marks it as a coprozoic rather than a truly parasitic amoeba, and eliminates it from the genera *Entamoeba* and *Endolimax*. It is not a *Hartmanella,* since it forms no spindle in nuclear division, and not a *Vahlkampfia,* since it has no polar caps. On the contrary, nuclear division seems to be accomplished through a direct amitotic division of the karyosome and then of the nucleus. At no time is a concentration of chromatin granules found arranged in an equatorial plate. Multinucleate cysts also distinguish it from *Vahlkampfia.*

Since this amoeba is found living coprozoically in the salamander, *Triturus viridescens,* it is proposed that it be called *Copramoeba salamandrae,* and that this species be the type species for the genus *Copramoeba,* with the characteristics as described above, and here summarized: nucleus with homogeneous central karyosome and peripheral chromatin, direct nuclear division without spindle or polar caps, contractile vacuole, and multinucleate cysts with the emergence of uninucleate forms.

SUMMARY

1. *Entamoeba pyrrhogaster* n. sp. was isolated from the intestine of the Japanese salamander, *Triturus pyrrhogaster,* and grown in culture, with encystation and excystation.

2. The trophozoite measured from 7 to 40 μ in diameter, with a few as large as 150 by 90 μ. The nucleus was histolytica-like with a peripheral rim of chromatin granules and a fine central karyosome.

3. Normally cysts measured from 5 to 10 μ and contained one to four nuclei; however, a few larger ones with as many as 16 nuclei were observed.

4. Metacystic amoebae emerged as quadrinucleate forms, in which each nucleus divided once producing eight. Cytoplasmic division followed nuclear division closely, so that metacystic amoebae with more than five nuclei were not found.

5. Attempts to infect *Rana pipiens,* a normal host of *Entamoeba ranarum,* with this *Entamoeba* were unsuccessful.

6. *Vahlkampfia salamandrae* n. sp. was recovered from *Triturus pyrrhogaster* and grown is culture.

7. The trophozoite, about 17 μ in diameter, had a typical *Vahlkampfia* nucleus with large central karyosome, and fine peripheral chromatin. Nuclear division was promitotic with the formation of polar caps, and an equatorial plate of chromatin granules.

8. Uninucleate cysts with smooth outer and inner walls, 13 μ in diameter, were readily formed. Excysting amoebae emerged through one of a number of pores in the cyst wall.

9. A third amoeba, *Copramoeba salamandrae* n. g. et sp., was found in *Triturus viridescens,* and grown in culture.

10. This amoeba was smaller than *Vahlkampfia,* 4 to 14 μ, but similar to it in general appearance. Ectoplasm was distinct from endoplasm only in formation of pseudopodia. A contractile vacuole was present.

11. The nucleus consisted of a dense spherical karyosome surrounded by a clear area and a nuclear membrane. Fine chromatin granules could be seen on the nuclear membrane, while linin threads sometimes were seen connecting it to the karyosome. In nuclear division there was no spindle or promitotic figure, but a direct division of the karyosome followed by nuclear division.

12. Cysts had thick walls, smoothly spherical on the inside and sticky and slightly irregular on the outside. The nucleus divided in the cyst. It is believed that the amoebae emerged as uninucleate forms.

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Explanation of Plates

All figures on Plates I–V were drawn with the aid of camera lucida from slides of culture material fixed in Schaudinn's fluid at room temperature and stained with Heidenhain's haematoxylin. Magnification is approximately × 1550.

Plate I

Entamoeba pyrrhogaster

Fig. 1. Small uninucleate trophozoite.
Fig. 2. Largest uninucleate trophozoite found on slides. Many starch grains and one cyst have been ingested.
Fig. 3. Trophozoite with elongating nucleus.
Fig. 4. Trophozoite with dividing nucleus, showing two pairs of chromosome-like granules.

PLATE I

Plate II

Entamoeba pyrrhogaster

Fig. 5. Large binucleate trophozoite with four ingested cysts.
Fig. 6. Uninucleate trophozoite.
Fig. 7. Small uninucleate trophozoite with dark food inclusions.
Fig. 8. Binucleate trophozoite.
Fig. 9. Dividing binucleate trophozoite.
Fig. 10. Small precystic amoeba devoid of food inclusions.
Fig. 11. Uninucleate cyst with large glycogen vacuole and chromatoidal bodies. Nucleus elongated prior to division.
Figs. 12–14. Uninucleate cysts with dividing nuclei.
Fig. 15. Binucleate cyst with nuclei still drawn out after division.
Figs. 16–18. Binucleate cysts; beginning of division in Fig. 18.
Figs. 19–22. Quadrinucleate cysts.

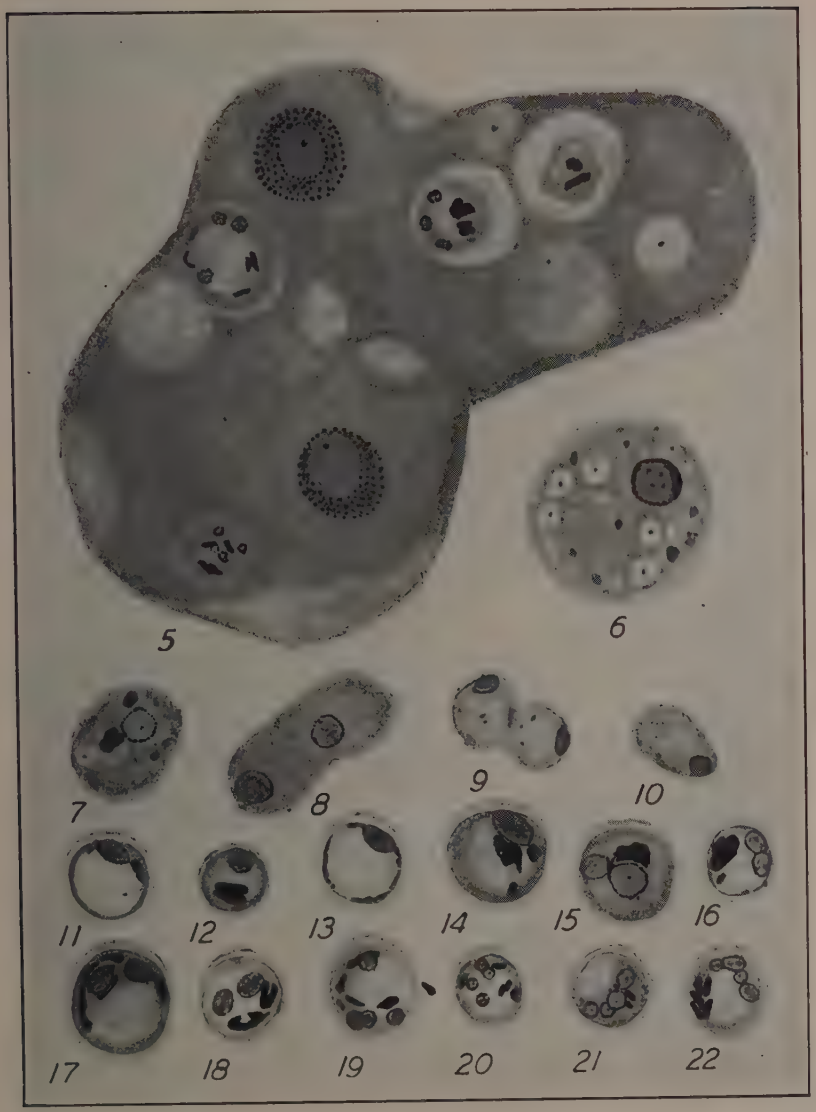

PLATE II

Plate III

Entamoeba pyrrhogaster

Fig. 23. Quadrinucleate cyst.
Fig. 24. Quadrinucleate cyst with one nucleus beginning to divide.
Figs. 25–26. Cysts with eight nuclei of various sizes. Normally only four nuclei are formed.
Fig. 27. Large cyst with at least 15 nuclei; rarely found.
Fig. 28. Mature cyst without glycogen vacuole and with only a little chromatoidal material remaining.
Figs. 29–46. Metacystic amoebae.
Fig. 29. With four cystic nuclei, NNNN. A few bacteria have been ingested.
Figs. 30–31. Quadrinucleate amoebae which have ingested many bacteria and have increased in size. Note increase also in size of nuclei.
Fig. 32. With four cystic nuclei, NNNN, one in the process of division.
Fig. 33. With three cystic and one metacystic nuclei, NNNn.
Figs. 34–35. With three cystic nuclei, NNN.
Fig. 36. With two cystic and two metacystic nuclei, NNnn.
Fig. 37. With two cystic and three metacystic nuclei, NNnnn.
Fig. 38. With two cystic and one metacystic nuclei, NNn.
Fig. 39. With one cystic and three metacystic nuclei, Nnnn.
Fig. 40. With one cystic and two metacystic nuclei, Nnn.
Fig. 41. With three metacystic nuclei, nnn.
Fig. 42. Wtih four metacystic nuclei, nnnn.
Fig. 43. With three metacystic nuclei, nnn.
Figs. 44–45. With two metacystic nuclei, nn.
Fig. 46. With one metacystic nucleus, n.

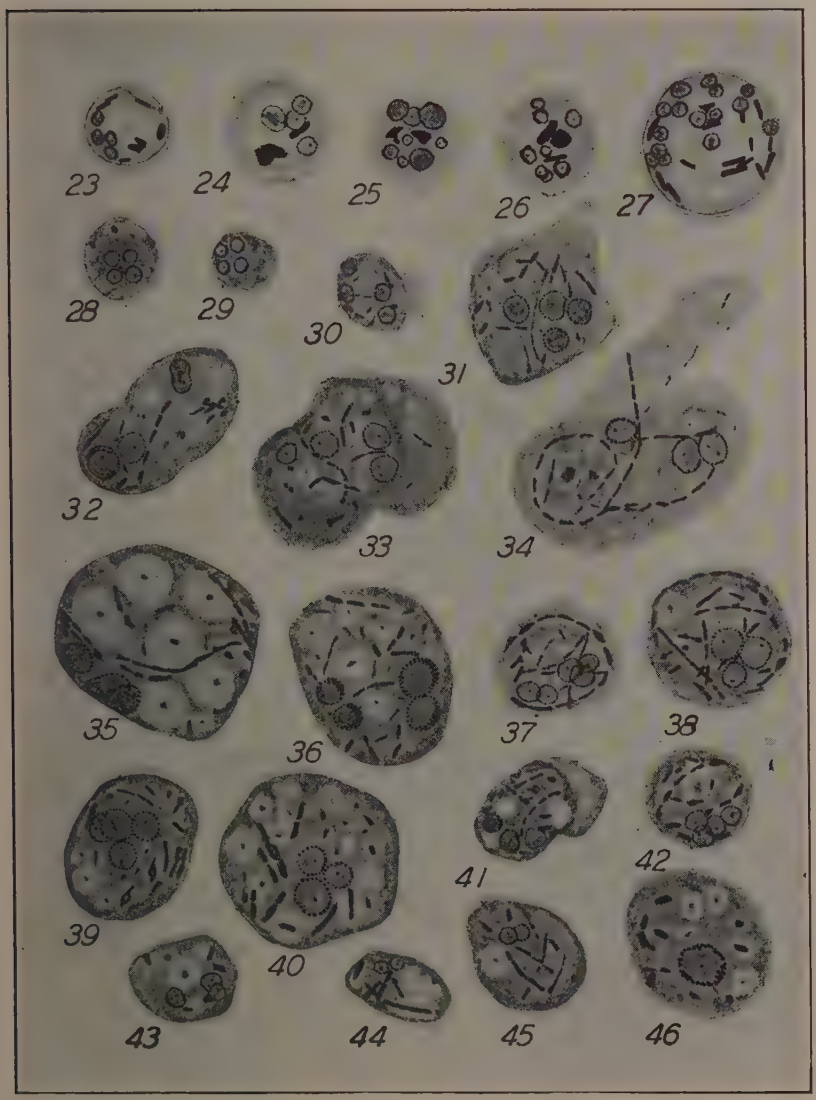

PLATE III

Plate IV

Vahlkampfia salamandrae

Fig. 47. Trophozoite with large mass of ingested bacteria.
Figs. 48–51. Uninucleate trophozoites showing variation in appearance of the nucleus.
Fig. 52. Trophozoite showing accumulation of chromatin material around a deeply staining karyosome in preparation for division.
Fig. 53. Trophozoite with only dark karyosome showing in nucleus.
Figs. 54–57. Successive stages in formation of equatorial plate with polar caps, by the elongation and division of the karyosome.
Fig. 58. Amoeba showing metaphase stage in nuclear division with chromatin arranged in equatorial plate.
Fig. 59. Early anaphase stage.
Fig. 60. Probably the telophase stage.
Fig. 61. Binucleate amoeba.
Fig. 62. Division of binucleate into two uninucleate amoebae.
Fig. 63. Division of quadrinucleate amoeba into two binucleate ones. Here nuclear division occurred more rapidly than cytoplasmic division.
Figs. 64–68. Uninucleate cysts. Fig. 66 shows pores and bacteria adhering to the outside of the cyst wall.
Fig. 69. Empty cyst, showing pores.
Fig. 70. Amoeba from enflagellating culture on agar plate, showing granule splitting off from the karyosome, to become the basal granule.
Figs. 71–72. Flagellated forms from plate cultures, showing two flagella arising from a single basal granule.

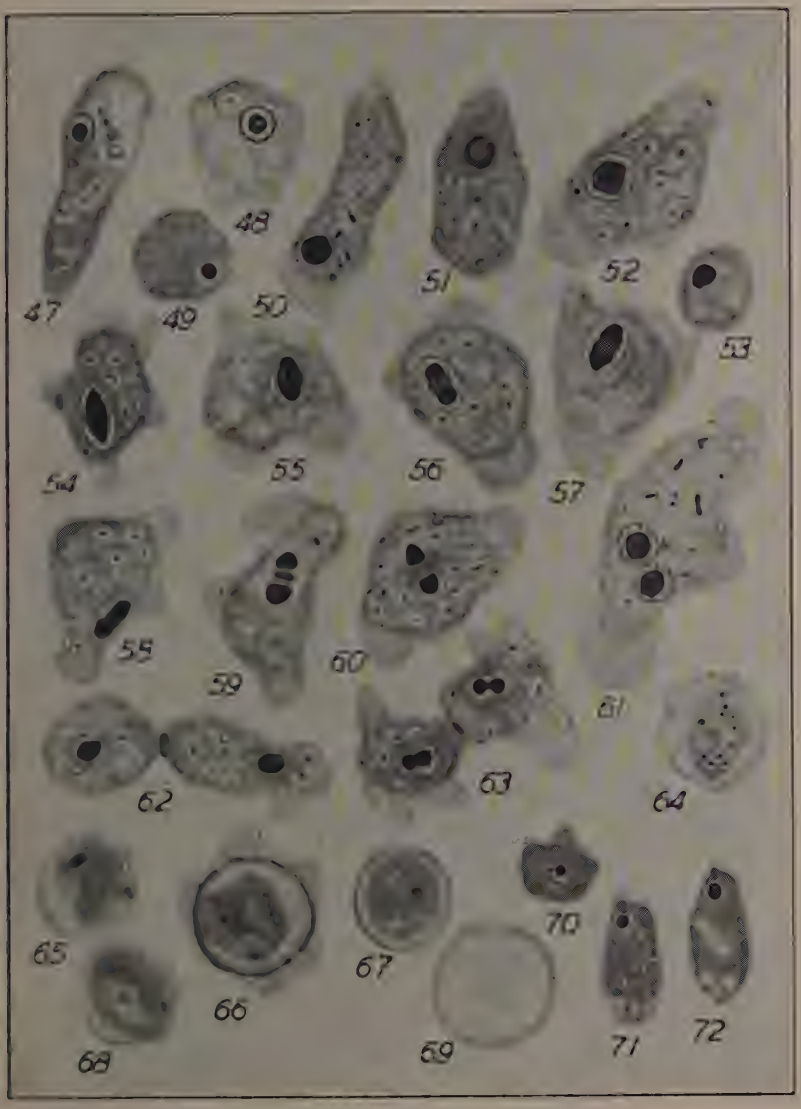
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PLATE IV

Plate V

Copramoeba salamandrae

Fig. 73. Trophozoite showing contractile vacuole, homogeneous central karyosome, peripheral chromatin and threads connecting karyosome with nuclear membrane.
Figs. 74–79. Trophozoites showing variation in appearance of nucleus.
Fig. 80. Trophozoite in preparation for division; only central part of karyosome stains deeply.
Figs. 81–82. Central part of karyosome elongating.
Fig. 83. Central part of karyosome almost entirely divided.
Figs. 84–86. Central part of karyosome divided into two.
Fig. 87. All of karyosome divided.
Figs. 88–90. Binucleate trophozoites.
Fig. 91. Binucleate trophozoite with two dividing nuclei.
Figs. 92–93. Apparent extrusion of karyosome from nucleus and cytoplasm, perhaps explaining the presence of some anucleate forms.
Figs. 94–97. Uninucleate cysts.
Fig. 98. Binucleate cyst.
Fig. 99. Cyst with one large and two small nuclei.
Fig. 100. Cyst with three small nuclei.
Figs. 101–103. Cysts with one large and three small nuclei.
Fig. 104. Cyst with one large and four small nuclei.
Fig. 105. Cyst with five small dark nuclei, and one small light one.
Figs. 106–107. Cysts showing differences in staining affinity of the nuclei, some light, some dark, and some intermediate. Cyst walls are irregular from crowding with other cysts.
Fig. 108. Cyst showing structures that may be small amoebae; with bacteria adherent to the outside.
Fig. 109. Empty cyst with small amoebae (?) outside at one end.

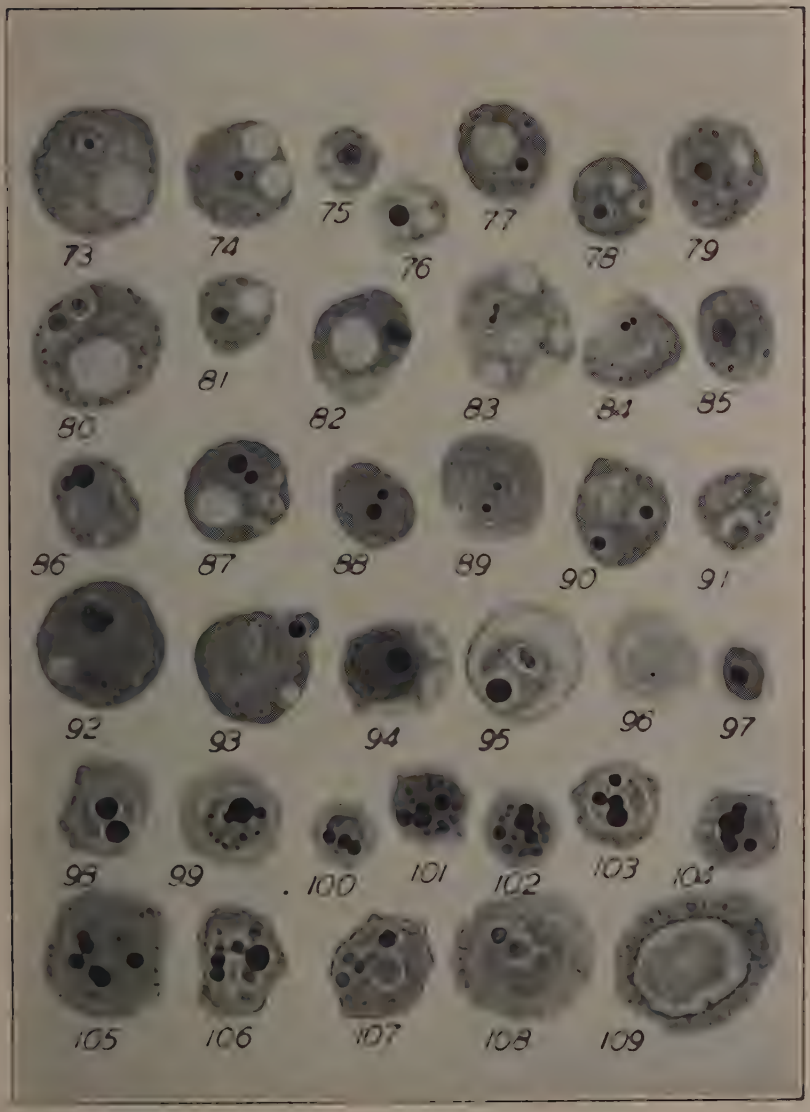
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PLATE V

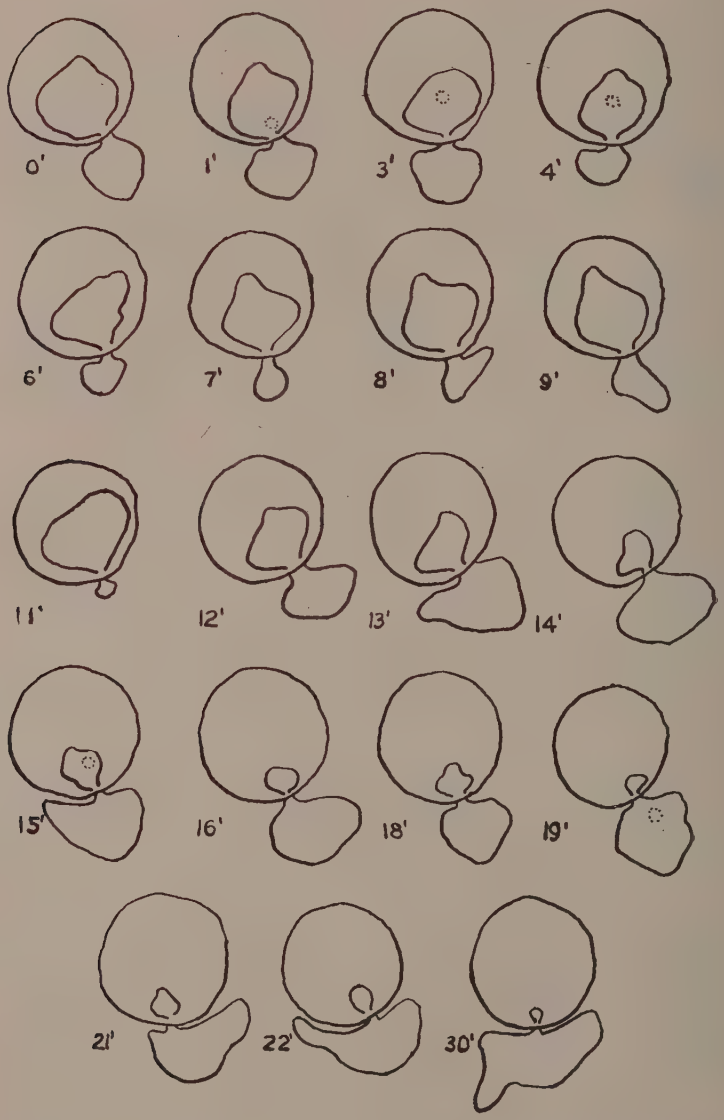

PLATE VI. Excystation of *Vahlkampfia,* showing movements of the amoeba for 30 minutes during emergence from the cyst. Drawn with camera lucida from living material.

HAEMOPROTEUS METCHNIKOVI (SIMOND, 1901) FROM THE YELLOW-BELLIED TERRAPIN (*PSEUDEMYS ELEGANS*)

REDGINAL HEWITT*

Comparatively little is known concerning the distribution and life cycles of the haemosporidia in cold-blooded animals. The first member of this group of intracellular blood protozoa to be described from reptiles was *Haemoproteus* (*Haemamoeba*) *metchnikovi,* found by Simond (1901) in the Indian river tortoise (*Trionyx indicus*). *Haemoproteus* and *Plasmodium* were later reported from lizards and snakes in various parts of the world. Laveran (1905), Johnston and Cleland (1911), Pittaluga (1912), Plimmer (1912), and others, found pigmented parasites in the red blood cells of turtles and tortoises from South Africa, West Africa, Australia, British Honduras, and New Guinea. Plimmer also found *Haemoproteus* in a painted terrapin (*Chrysemys picta*) from North America which died in the London Zoological Gardens. Although all of these parasites in turtles were given different names (Table 1), the descriptions in each case are similar and Wenyon (1926) is of the opinion that they all belong to a single species, *Haemoproteus metchnikovi* (Simond, 1901).

In the present paper *H. metchnikovi* is described from *Pseudemys elegans,* an inhabitant of the eastern Atlantic seaboard sold in the market under the name of yellow-bellied terrapin. The infected terrapin was obtained through the courtesy of Miss Alla Iverson of the Department of Zoology, the Johns Hopkins University, who recognized parasites in the blood cells and brought a slide to the writer for identification. Five other terrapins belonging to the same species were examined with negative results.

As mentioned above, Plimmer (1912) reported pigmented parasites in turtles from North and Central America which died in the London Zoological Gardens, but it is not definitely known whether these infections were contracted on this side of the Atlantic Ocean. No other accounts of *Haemoproteus* in American turtles have come to the writer's attention.

DESCRIPTION

In every respect the parasite found in *Pseudemys elegans* agrees with the description given by Simond (1901) of *Haemoproteus* (*Haemamoeba*) *metchnikovi* in the Indian river tortoise. Sexual forms only occur in

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TABLE 1.—*Host records of* Haemoproteus metchnikovi

Species of turtle	Locality	Name given	Author
Trionyx indicus	India	*Haemamoeba metchnikovi*	Simond, 1901
Testudo pardalis	South Africa	*Haemamoeba testudinis*	Laveran, 1905
Cinixys belliana	West Africa	*Plasmodium roumei* *Haemocystidium* sp. *Haemocystidium* sp.	Bouet, 1909[1] Plimmer, 1912 Joyeux, 1913[1]
Chelodina longicollis	Australia	*Haemocystidium chelodinae*	Johnston and Cleland, 1909[1]
Emydura krefftii	Australia	" "	" " " 1910[1]
Chelodina oblonga	Australia	" "	" " " "
Clemmys africana	New Guinea	*Haemoproteus cajali*	Pittaluga, 1912
Staurotypus triporcatus	British Honduras	*Haemocystidium* sp.	Plimmer, 1912
Chrysemys picta	North America	" "	" "
Cinixys homeana	West Africa	" "	" "
Cinixys erosa	West Africa	" "	" "
Pseudemys elegans	Eastern United States	*Haemoproteus metchnikovi*	Hewitt, 1940

[1] References quoted by Wenyon (1926) but not read.

the peripheral blood cells and mature male and female gametocytes are easily differentiated. Young gametocytes in various stages of development may be found. Approximately 4 per cent of the red cells were infected in the present case, and many double, triple, and quadruple infections occurred.

Both the male and female gametocytes generally occupy a polar position within the cell and show various irregularities in shape (Plate I). Mature forms bend around one of the terminal ends of the nucleus, extending down about 1/3 the length of the red cell on either side in most cases. Round forms have been found. In few cases is the nucleus displaced or the host cell hypertrophied.

The female gametocyte stains deeply with Giemsa's stain and the cytoplasm appears distinctly vacuolated. The nucleus is compact and is most frequently located near the center of the parasite, but variations in this respect occur. Short, rod-shaped pigment granules are clumped in various parts of the parasite, the usual number being about 20. Measurements made of 25 mature female gametocytes averaged 9.5 micra in length by 5.8 micra in width.

The male gametocyte is similar in the above respects, with the exception that the cytoplasm stains pink and the nucleus is dispersed throughout the center of the parasite. The average size (from 25 measured specimens) is 9 micra long by 5.3 micra wide.

As shown in Plate 1 the shape of the gametocytes is quite variable and the round forms sometimes encountered resemble the gametocytes of *Plasmodium relictum* in birds. However, the slightly bent, reniform gametocytes, occupying a polar position within the host cell seem to be most typical of *H. metchnikovi* (Figs. 1 and 4).

EXAMINATIONS OF THE VISCERAL ORGANS

Smears and sections from different parts of the spleen, liver, and lung showed no asexual stages. A satisfactory preparation from the bone marrow could not be obtained. It is of interest that the asexual stages of *Haemoproteus* in turtles have never been found, and the life cycle is entirely unknown. The leech is suspected as the transmitting host (Wenyon, 1926), but development of the parasite in this invertebrate host has not been demonstrated.

DISCUSSION

A striking fact concerning *Haemoproteus* found in turtles is that the species reported from different turtles throughout the world (Table 1) appear to be exactly the same. The descriptions of Simond, Laveran, Johnston and Cleland, and Pittaluga agree in every respect with the observations herein reported, even though the species of host and the

locality from which they were obtained are widely different. Two interpretations for this might be offered. In the first place comparatively few records of *Haemoproteus* in turtles exist, and the same species may have been found in the turtles which have been examined purely by coincidence. Many more studies will have to be made on the intracorpuscular parasites of turtles before it will be known whether or not other species of *Haemoproteus* occur in this group of reptiles. Another explanation may be that the species of *Haemoproteus* found in turtles thus far represents a form more like the undifferentiated ancestral type than any species yet reported from warm-blooded vertebrates. The evolutionary development of parasitic protozoa may never be clearly understood, since, as Huff (1938) points out, fossil records of this group will probably not be recognized. Furthermore, it is not known whether mammals, birds, and reptiles acquired their blood protozoa at approximately the same time in geologic history or whether the parasites developed along with their hosts. It is interesting to speculate, however, that *Haemoproteus metchnikovi* represents a species which has undergone little morphological change in the various species of turtles from which it has been reported in different parts of the world simply because it inhabits the red cells of an animal in which the undifferentiated ancestral type might have occurred. Since pigmented parasites have not as yet been reported from amphibians or fish it is reasonable to assume that the reptiles were among the first to harbor them.

SUMMARY

1. *Haemoproteus metchnikovi* (Simond, 1901) is described from a yellow-bellied terrapin (*Pseudemys elegans*) obtained from a sea food market in Baltimore, Maryland.

2. Sexual stages only occurred in the blood and preparations made from the lung, spleen, and liver did not reveal segmenting forms.

3. It is believed that this may be considered the first record of *Haemoproteus* from an American turtle, since the only other record from this hemisphere is that of Plimmer (1912) in which case it is not definitely known whether the turtles acquired the infection in America or in the London Zoological Gardens.

4. Since all species of *Haemoproteus* which have been described from various turtles in different parts of the world are thus far probably the same it is suggested that *H. metchnikovi* may represent a form more like the ancestral type than any species yet found in warm-blooded vertebrates.

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EXPLANATION OF PLATE, P. 278.

Haemoproteus metchnikovi (Simond, 1901) from the yellow-bellied terrapin.

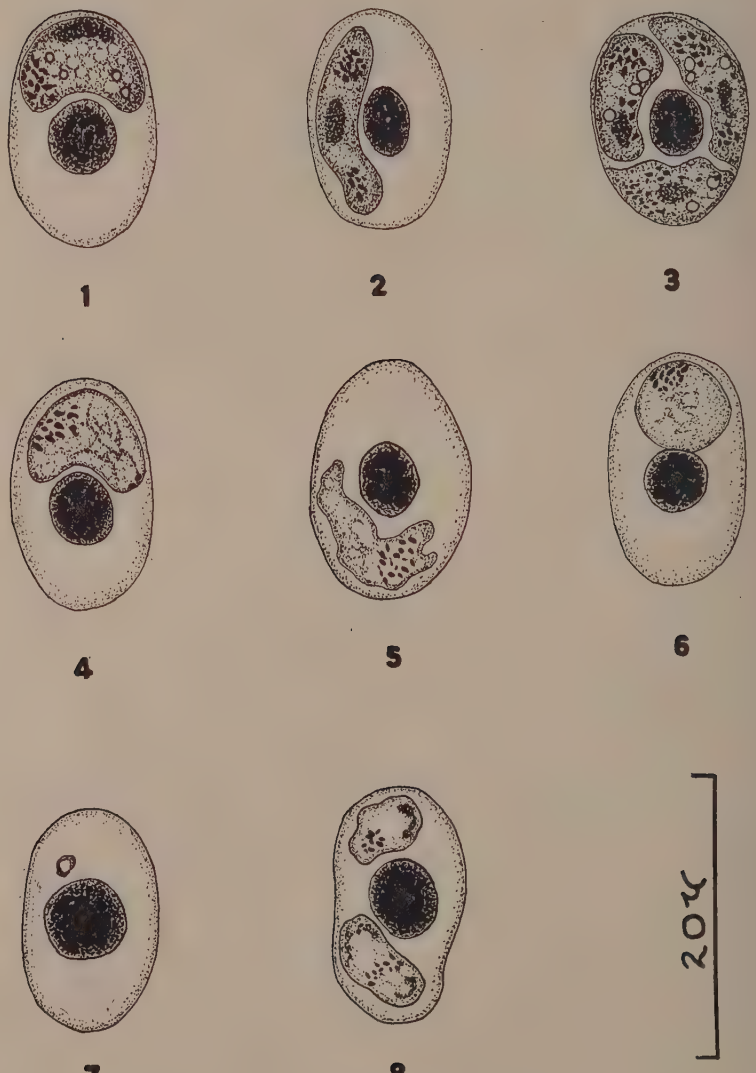
FIGS. 1 to 3. Female gametocytes. Fig. 1 represents the typical, reniform gametocyte. Fig. 3 is a multiple-infected cell.

FIGS. 4 to 6. Male gametocytes.

FIG. 7. Ring stage.

FIG. 8. Two immature gametocytes in a single red cell.

All figures drawn with the aid of the camera lucida from Giemsa preparations of the peripheral blood.



Haemoproteus metchnikovi (Simond, 1901)

DESCRIPTION OF THREE NEW TREMATODES WITH NOTES ON OTHER SPECIES OF *ACANTHATRIUM* (LECITHODENDRIIDAE), AND A KEY TO THE GENUS

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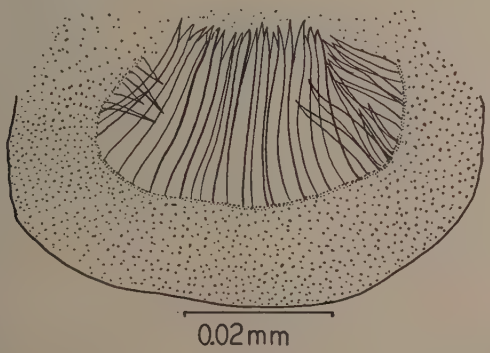
Trematodes of the genus *Acanthatrium* Faust, 1919, have often been encountered in Minnesota bats dissected by the writer, although they have not been found to be as numerous in individuals as in the case of other lecithodendriids. Three new species are described in this paper. For the loan of type and paratype material of *A. alicatai* and other species I express my thanks to Dr. E. C. Faust, Dr. J. E. Alicata, Dr. W. E. Martin, and Dr. E. W. Price.

Three specimens of the first species were obtained for study from a pipistrelle bat taken in hibernation at St. Peter, Minn., February 12, 1934. The species is characterized by the single group of long, slender spines of the genital atrium, each spine measuring about 25 μ long; by the subequal suckers which are large in proportion to the size of the body, and by the short space between them.



TEXT-FIG. 1. *Acanthatrium pipistrelli*, atrial spines of type, enlarged.

Acanthatrium pipistrelli n. sp.

(Fig. 1, Text-Fig. 1)

Specific diagnosis: Acanthatrium: Body oval to elliptical, 0.60 mm long by 0.48 mm wide. Cuticula without spines. Oral sucker 0.10 to 0.12 mm in diameter, subterminal. Acetabulum 0.12 to 0.13 mm wide by 0.09 to 0.10 mm long, located about midway between the testes and only a short distance from the oral sucker. Pharynx 0.040 mm wide by 0.026 mm long. Esophagus extremely short. Intestinal ceca very short but reaching testes. Testes 0.10 to 0.12 mm in diameter, lateral to acetabulum

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and prostate mass. Prostate mass 0.13 to 0.16 mm in diameter; anterior margin touching or close to ceca overlapped by acetabulum. Spines of the genital atrium in a single compact group in the anterior part of the prostate mass, directed posteriad; about 35 in number. The spines measure about 25 μ long. Ovary small, ovoid, entire; 0.07 mm wide by 0.05 mm long. Vitellaria in compact lateral masses, reaching from the level of the pharynx to the testes. Uterus filling the body posterior to the testes and acetabulum. Egg 13 μ by 24 μ in size.

Host: Pipistrellus subflavus (F. Cuvier).

Location: Small intestine.

Locality: St. Peter, Minnesota.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 36670; paratypes in author's collection.

The second species has been found on two occasions in hibernating bats; three specimens were taken from a big brown bat, March 5, 1936, and three others were collected previously from the same kind of bat. The description follows:

Acanthatrium microcanthum n. sp.

(Figs. 7, 8, 10, 11)

Specific diagnosis: Acanthatrium: Body 0.9 to 1.1 mm long by 0.65 to 0.70 mm wide. Cuticula aspinose. Oral sucker 0.12 to 0.16 mm wide by 0.07 to 0.15 mm long, subterminal. Ventral sucker 0.12 to 0.15 mm wide by 0.08 to 0.14 mm long, located between the testes and overlapping the prostate mass. Pharynx nearly spherical, from 0.04 to 0.06 mm in diameter. Esophagus in relaxed specimens very long. Intestinal ceca of the short lecithodendriid type. Testes rounded, 0.19 mm in diameter, postequatorial in relaxed specimens and equatorial to pre-equatorial in contracted examples. Prostate mass averages about 0.17 mm in diameter and is situated between the testes and the intestinal fork. The genital atrium is near the middle or in the anterior part of the prostate mass. Spines which line the atrium in a single row, measure 3 μ to 7 μ in length. A coiled seminal vesicle is present. Ovary about 0.15 mm in diameter; between the testes. In contracted specimens it is shifted slightly caudad. Vitellaria reduced, located around the intestinal ceca, just anterior to the testes. Follicles of the vitellaria rather distinct; from 5 to 8 on each side. Uterus with heavy slings nearly filling the posterior part of the body. In living specimens, the portion of the uterus leading into the metraterm is greatly swollen and much darker than the rest of the uterus due to the change in egg color. Slings of uterus tend to be transverse. Eggs measure 14 μ to 16 μ by 23 μ to 27 μ.

Host: Eptesicus fuscus (Beauvois).

Location: Small intestine.

Locality: St. Paul, Minnesota.

Type specimens: U. S. Nat. Mus. Helm. Coll. No. 36669; paratypes in author's collection.

Due to the smaller number and size of spines in three specimens of *A. microcanthum* it was at first believed two species were involved. However, it is probable that in these examples some of the spines were turned up and therefore not recognizable.

In addition to the foregoing new species, one specimen of *A. eptesici* Alicata, 1932, was found in the intestine of *Eptesicus fuscus* in St. Paul, Minnesota. This specimen has the same arrangement of organs described for Alicata's species, but the ventral sucker is slightly larger in proportion to other structures than in *A. eptesici.* The atrial spines (Fig. 3)

are of the same size but have a slightly different arrangement with several spines facing the anterior group.

Through the courtesy of Dr. E. C. Faust, the writer was enabled to examine the type of *A. nycteridis* and found that the spines (Fig. 9) were quite different from those illustrated by Alicata, 1932, for that species. An examination of Alicata's two specimens, deposited in the National Museum, confirmed the belief that they represented an undescribed form. A description of this species follows.

Acanthatrium alicatai n. sp.

(Fig. 2)

Specific diagnosis: Acanthatrium: Body pyriform, nearly subtriangular, without cuticular spines, 0.65 to 0.81 mm wide by 0.65 to 0.89 mm long. Oral sucker terminal to subterminal, 0.09 to 0.10 mm wide by 0.09 mm long. Pharynx 0.04 to 0.05 mm in diameter. Esophagus of moderate length. Acetabulum 0.075 to 0.095 mm in diameter, situated between the testes and lying on the prostate mass. Testes 0.12 to 0.15 mm wide by 0.19 mm long. Prostate mass 0.20 to 0.23 mm in diameter, largely filling the space between the testes and the intestinal ceca. Genital atrium in the anterior part of the prostate mass; with three pockets each containing from 8 to 12 spines. The spines (Fig. 12), which are especially characteristic, are dull pointed, mostly curved and broad at the base, and measure 22 μ to 26 μ long. Ovary 0.15 mm wide by 0.12 mm long, slightly irregular in outline, its field overlapping the posterior part of the testicular zone. Vitellaria in compact masses along the anterior margins of the ceca; extending back to the testes. Uterus largely filling the body caudad of the testes. Eggs 17 μ to 18 μ by 29 μ to 32 μ long.

Host: Eptesicus fuscus (Beauvois).

Locality: Washington, D. C.

Type specimens: Type U. S. Nat. Mus. Helm. Coll. No. 30139; paratype No. 30138.

A. alicatai differs from the other species of the genus by the character of the spines which are unusually large and arranged in three facing groups. The spines of *A. nycteridis* are much shorter, 10 μ to 15 μ long, narrow and sharp, three times as numerous, and scattered.

In order to facilitate comparison, the description of *A. molossidis* has been expanded with the aid of a paratype loaned by Dr. W. E. Martin.

Acanthatrium molossidis Martin, 1934

(Figs. 4, 5, 6)

Body of specimen examined 0.92 mm long by 0.87 mm wide. Cuticula with minute spines on anterior third of body. Oral sucker subterminal, in size 0.10 mm wide by 0.11 mm long. Pharynx spherical, 0.05 mm in diameter. Esophagus not evident. Intestinal ceca similar to those of other species of the genus. Testes oval, 0.14 to 0.17 mm in diameter. Genital pore and atrium slightly to left of median plane and near acetabulum. Atrial spines small, about 5 μ long, few in number and directed cephalad. Ovary about 0.20 by 0.12 mm, situated between the acetabulum and testes and touching the prostate mass, 0.28 mm wide by 0.22 mm long, containing a coiled seminal vesicle. Vitellaria consists of large compact masses of follicles extending from the testes to the oral sucker and overlapping the intestinal ceca. Uterus fills the posterior part of the body. Eggs 30 μ to 35 μ long by 20 μ wide.

Host: Molossidis sinaloae Allen.

Location: Intestine.

Locality: Honduras.

This species differs from all others of the genus by the possession of cuticular spines, although they are very small and inconspicuous. The location of the genital atrium is distinctive since it is near the posterior part of the prostate mass, and has a few small spines which point cephalad.

Key to the Species of *Acanthatrium*

1. Ovary reaching as far cephalad as the anterior level of the prostate mass; deeply lobed. Spines of genital atrium scattered; numerous
Acanthatrium sphaerula (Looss), 1896
Ovary not extending so far cephalad 2
2. Genital atrium latero-posteriad in prostate mass. Spines of genital atrium about 15 in number, approximately 5 μ long, straight, and arrranged in a crescent-shaped row. Small cuticular spines present.
Acanthatrium molossidis Martin, 1934
Cuticular spines absent and other characters not as above 3
3. Spines of genital atrium about 25 μ long; either arranged in three facing groups or in a single compact crescentic group near the middle of the prostate mass 4
Spines of genital atrium much less than 25 μ long 5
4. Spines of genital atrium arranged in three facing groups; spines very broad at the base ..... *Acanthatrium alicatai* n. sp.
Spines of genital atrium arranged in a single compact crescentic group
Acanthatrium pipistrelli n. sp.
5. Atrial spines 3 μ to 7 μ long. Prostate mass smaller than either testis
Acanthatrium microcanthum n. sp.
Atrial spines longer. Prostate mass not smaller than either testis .. 6
6. Suckers approximately equal in size. Atrial spines 10 μ to 15 μ long, arranged in a single long crescent in the anterior part of the prostate mass ..... *Acanthatrium oregonense* Macy, 1939
Oral sucker at least one fifth larger than acetabulum. Atrial spines 10 μ to 18 μ long, not arranged in a single row 7
7. Testes definitely anterior to acetabulum. Genital atrium with numerous scattered spines 10 μ to 15 μ long.
Acanthatrium nycteridis Faust, 1919
Acetabulum in testicular zone. Genital atrium with anterior wall lined with spines 12 μ long and provided at its antero-sinistral corner with a blunt conical diverticulum measuring 33 μ by 27 μ and bearing a group of spines 18 μ long by 2 μ broad.
Acanthatrium ovale Yamaguti, 1939

The genus *Acanthatrium*, erected by Faust, 1919, for lecithodendriid flukes having spines in the genital atrium, now contains eight species and a subspecies, *A. nycteridis plicati* Bhalerao, 1926. It would be interesting to compare the spines of Bhalerao's examples with those of *A. nycteridis*. Examination of the spines of a total of more than one hundred specimens

of five species of the genus shows that they are quite constant in length and arrangement, which indicates that they are of much taxonomic value. It has been found advisable to use care in selecting spines for measurement as the apparent shortness of some is due actually to the fact that they are tipped at an angle.

The recent discovery by Yamaguti, 1939, of a trematode of another genus, *Lecithodendrium japonicum,* with spines in the genital atrium, throws some doubt on the validity of using such spines as a generic character.

At present little can be said as to the relationships of species. However, *A. pipistrelli* seems close to *A. eptesici* due to similarity of the size and arrangement of spines.

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Explanation of Plates

All drawings made with the aid of the camera lucida

Plate I

Fig. 1. *Acanthatrium pipistrelli,* type, dorsal aspect.
Fig. 2. *Acanthatrium alicatai,* type.
Fig. 3. *Acanthatrium eptesici,* atrial spines, enlarged.
Fig. 4. *Acanthatrium molossidis,* egg.
Fig. 5. *Acanthatrium molossidis,* paratype.
Fig. 6. *Acanthatrium molossidis,* atrial spines, enlarged.
Fig. 7. *Acanthatrium microcanthum,* group of atrial spines, enlarged.

Plate II

Fig. 8. *Acanthatrium microcanthum,* contracted specimen.
Fig. 9. *Acanthatrium nycteridis,* atrial spines from type, enlarged.
Fig. 10. *Acanthatrium microcanthum,* type, dorsal aspect.
Fig. 11. *Acanthatrium microcanthum,* atrial spines from type, enlarged.
Fig. 12. *Acanthatrium alicatai,* atrial spine, enlarged.

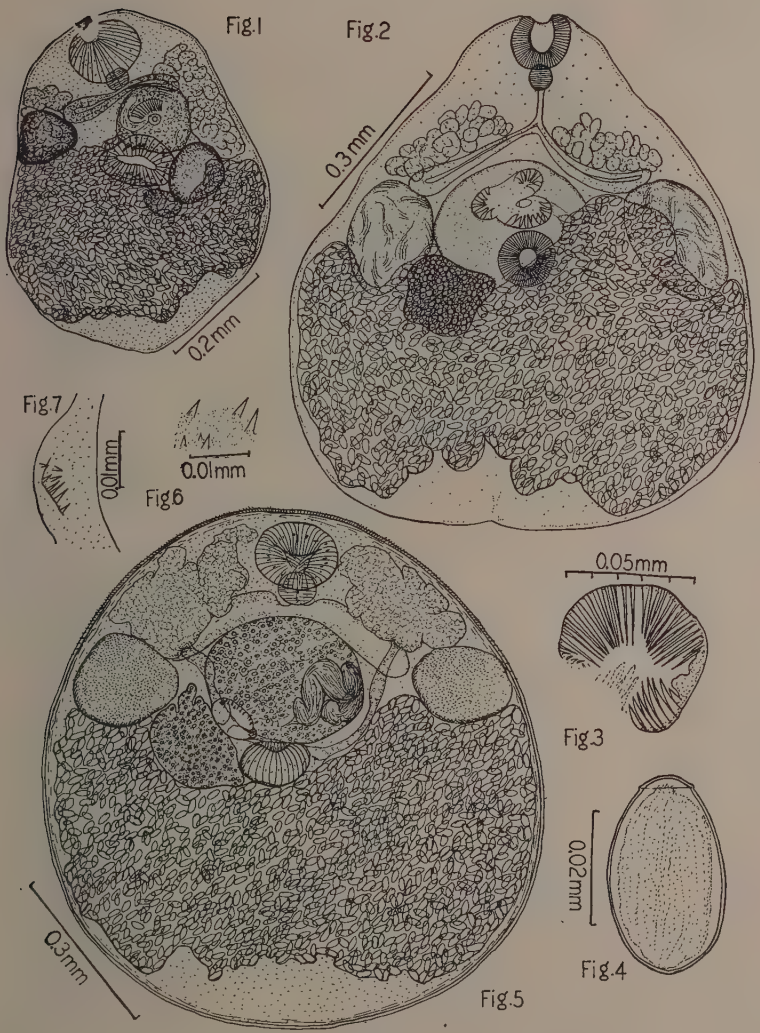
Fig.1
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PLATE I

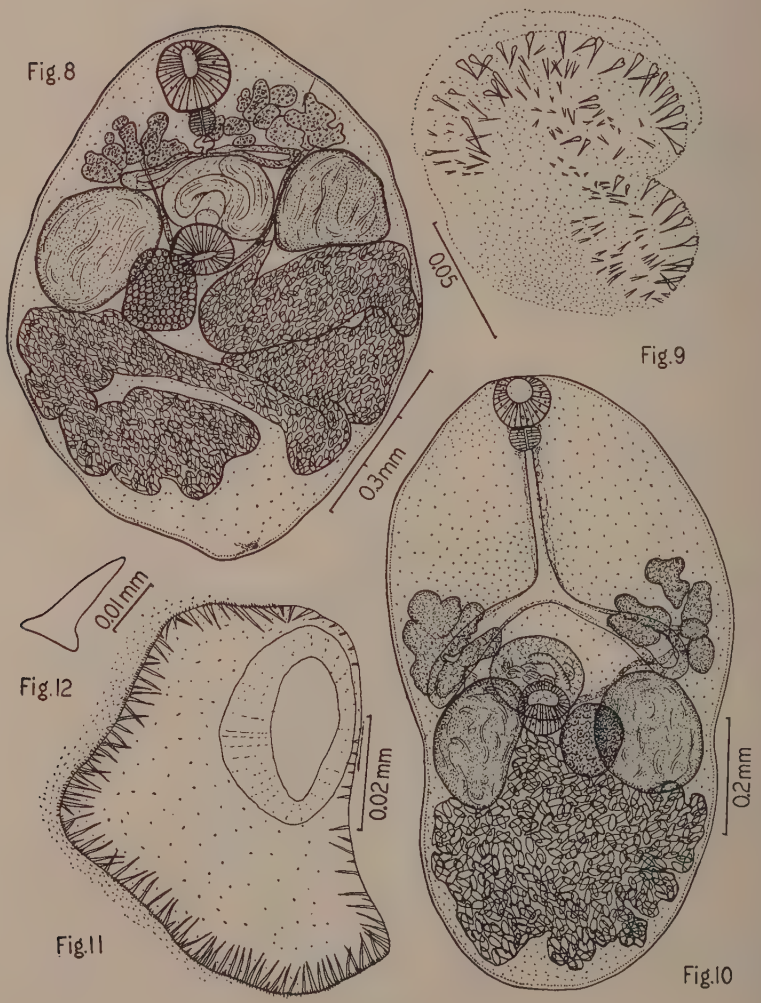

PLATE II

STUDIES ON BLOOD PROTOZOA OBTAINED FROM MEXICAN WILD BIRDS

REDGINAL HEWITT*

The material herein reported is a continuation of studies made by Beltrán (1939) on the blood protozoa of native birds from Mexico City and its environs. In his first report (1939) the above author examined 276 birds obtained from street markets, and in a later paper (1940) results are given from 85 birds, many of which were obtained in some of the suburban districts surrounding Mexico City (Texcoco, Xochimilco), and Toluca. Infections with *Plasmodium, Haemoproteus,* and *Leucocytozoon* were found. Sixty-one birds from which peripheral blood examinations had been previously made by Beltrán were given by him to the writer for more extended examination, and the remaining 33 birds included in the present paper were obtained from markets in Mexico City.

METHODS

As shown in Table 1, 3 species of birds were examined, 61 common house finches (*Carpodacus mexicanus frontalis*), 30 *Molothrus* sp., and 3 cedar waxwings (*Bombycilla cedrorum*). Peripheral blood smears and smears from the spleen, liver, bone marrow and brain were made from each bird. Saline preparations were made of the bone marrow of many birds since trypanosomes could be more easily found in wet mounts. In a few cases subinoculations were made to canaries. All blood and tissue smears were stained with Giemsa's stain.

In most surveys which have been made of avian blood protozoa peripheral blood smears only have been examined. Manwell and Herman (1935), however, supplemented this method by subinoculating blood from wild birds into parasite-free sparrows or canaries. They found that the number of positive cases, as compared with the examination of smears, was trebled in many instances when this method was used. They likewise examined smears of the internal organs of some of their birds. Herman (1938) made smears of the internal organs of many of the birds which he examined from the Cape Cod region. In the present case it was not possible to obtain a sufficient number of canaries to subinoculate with blood from each wild bird. Undoubtedly a higher incidence would have been discovered had such a procedure been followed. Many infections, however, were revealed from slides of the spleen, liver,

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TABLE 1.—*The incidence of blood parasites in certain Mexican wild birds*

Species of bird	Number examined	Plasmodium		Haemoproteus		Leucocytozoon		Trypanosoma		Unclassified intra-leucocytic parasites		Filaria	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Carpodacus mexicanus frontalis	61	10	16	13	21	4	6	17	28	20	32	2	3
Molothrus sp.	30	2	6	0	0	1	3	7	23	7	23	2	6
Bombycilla cedrorum	3	0	0	0	0	2	67	1	33	0	0	0	0
Total	94	12	12	13	13	7	7	25	25	27	27	4	4

or bone marrow which were not found in peripheral blood smears. This is particularly true of the trypanosomes found in all three species of birds examined.

INCIDENCE

Of the 94 birds examined, 12 infections with *Plasmodium,* 13 with *Haemoproteus,* 7 with *Leucocytozoon,* 25 with *Trypanosoma,* 27 with intra-leucocytic parasites not definitely identified, and 4 with microfilariae were found. Data are presented in Table 1. Twenty-one birds exhibited mixed infections with two or more blood parasites, the combination most frequently encountered being *Plasmodium* or *Haemoproteus* in the peripheral blood and *Trypanosoma* in the bone marrow. Six infections with *Leucocytozoon,* 3 with *Plasmodium,* 1 with *Haemoproteus* and all of the other types of blood protozoa listed in Table 1 were diagnosed from smears of the internal organs without being found in the peripheral blood.

Plasmodium

Nine of the finches were infected with *Plasmodium relictum* and one finch showed *P. cathemerium. P. relictum* was also found in one *Molothrus* sp. Quite a different plasmodium was found in another *Molothrus.* This was a small parasite generally localized at the ends of the host cell near the nucleus, many times in direct contact with the nucleus. The number of merozoites in what seemed to be mature segmenters averaged from 5 to 7. No gametocytes were found. Of avian plasmodia which have been described this parasite appears more like *P. nucleophilum* than any other, but since subinoculations were not made a definite classification is not attempted on morphological characteristics alone. Sub-inoculations were made to canaries from three of the finches which showed plasmodia in their peripheral blood. In two canaries infections with *P. relictum* were obtained and the third canary showed *P. cathemerium.* In the *P. relictum* infections segmentation was matinal, occurring between 9:00 AM and 1:00 PM. Exo-erythrocytic bodies were found in canaries carrying the strain of *P. cathemerium* originally isolated from the finch. Separate papers will be devoted to studies of these two species of avian plasmodia with regard to their behavior in canaries.

Haemoproteus

The only previous record of *Haemoproteus* being found in common house finches (*Carpodacus mexicanus frontalis*) outside of Mexico is that of Wood and Wood (1937) from five birds in the vicinity of Los Angeles. They do not give detailed descriptions of the parasite, but include four illustrations showing male and female gametocytes. In the present case the gametocytes of *Haemoproteus* found in all 13 common

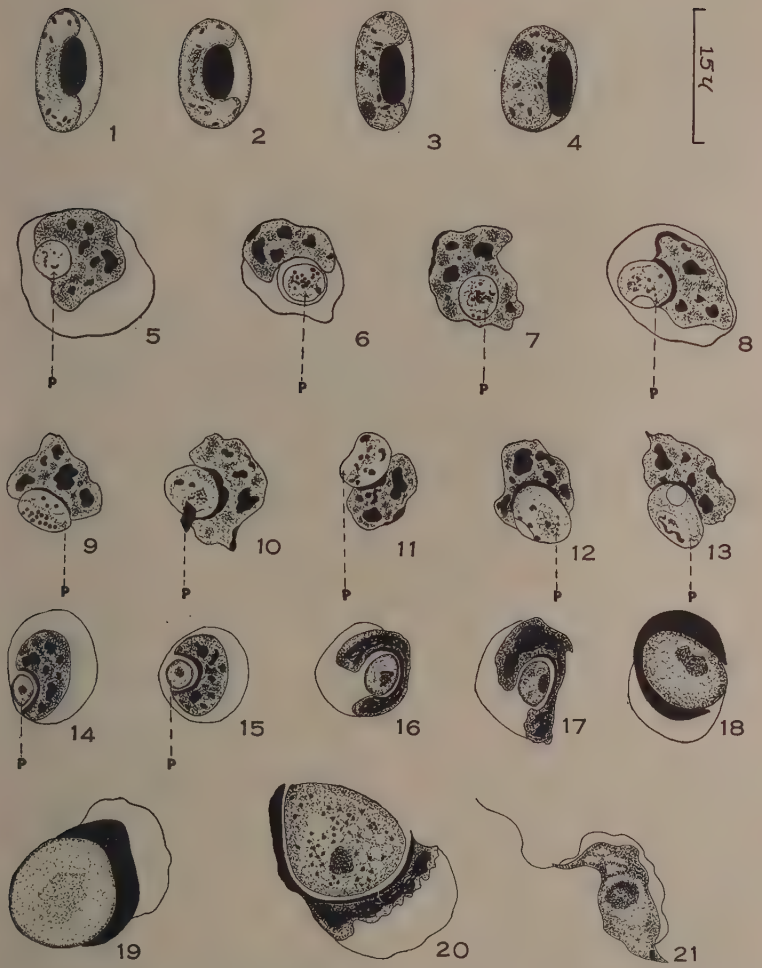
house finches were morphologically similar in every bird. Both male and female gametocytes occupy about two-thirds of the red cell, curving around the host cell nucleus at either end and often displacing the host cell nucleus (Figs. 1–4). The ends of both types of gametocytes are rounded. From 8 to 15 rod shaped pigment granules are present. The female gametocyte measures 11.6 micra in length by 2.8 micra in width (mean) at the center. The nucleus of the female gametocyte is eccentric and generally lies on the side near the outer wall of the red cell at one extremity of the parasite or the other. Its mean measurement is 2.8 micra long by 1.7 micra wide. The nucleus of the male gametocyte is dispersed throughout the center of the organism and shows great variation in size and position. The female gametocyte stains darker than the male.

Haemoproteus was not found in the *Molothrus* sp. or in the cedar waxwings.

Leucocytozoon

Parasites belonging to this genus were found in 4 of the finches, 1 *Molothrus* sp. and in 2 cedar waxwings. In all three species of birds in which the parasite was found the measurements and general appearance of the male and female gametocytes were the same. Both kinds of gametocytes were of the oval type without spindle-shaped projections to the nucleus of the host cell (Figs. 19 and 20). The mean measurements of the female gametocytes are 10.8 micra long by 6.5 micra wide, and of the male 12.1 micra long by 8.3 micra wide. The nucleus of the female gametocyte shows a mean diameter of 3.1 micra and is usually eccentric in its position within the parasite. The nucleus of the male gametocyte is diffuse and variable in its extent. The nucleus of the host cell generally surrounds from one-half to two-thirds of the parasite but rarely surrounds more than one-half of the parasite. A number of deeply-staining granules are frequently present in either the male or female gametocyte but they show no constancy in size or distribution. The female gametocyte stains more deeply than the male.

Immature stages of Leucocytozoon. In smears of the brain and bone marrow of some of the birds very young stages of *Leucocytozoon* were found in immature red cells (Figs. 14, 15, 16, 17). *Leucocytozoon* received its name from the supposed fact that it inhabited leucocytes. A more recent belief, however, by Wenyon (1926), is that the parasites penetrate immature red cells. In the present case, a series of stages was found in the brain and bone marrow which demonstrate this fact. In Fig. 14, for example, a very young *Leucocytozoon* is located in a concavity of the nucleus of a polychromatophilic erythroblast. Figs. 15, 16, and 17 show progressively larger stages, with greater distortion of the host-cell nucleus as the parasite continues to enlarge. These



FIGS. 1 and 2. Male gametocytes of *Haemoproteus* sp. from the common house finch (*Carpodacus mexicanus frontalis*).

FIGS. 3 and 4. Female gametocytes of *Haemoproteus* sp. from the common house finch.

FIGS. 5 to 13. An unclassified intra-leucocytic parasite found in the spleen, liver, and bone marrow of three species of Mexican birds. The large, irregular-shaped nucleus of the leucocyte usually lies in direct contact with the parasite. The host-cell membrane is not always visible.

FIGS. 14 to 17. Immature stages of *Leucocytozoon* sp. in erythroblasts from the brain and bone marrow of the cedar waxwing (*Bombycilla cedrorum*).

FIG. 18. A small female gametocyte of *Leucocytozoon* sp. from *Molothrus* sp.

FIG. 19. Mature male gametocyte of *Leucocytozoon* sp. from *Molothrus* sp.

FIG. 20. Mature female gametocyte of *Leucocytozoon* sp. from *Molothrus* sp.

FIG. 21. A trypanosome from the bone marrow of *Molothrus* sp.

All figures drawn with aid of camera lucida. Letter p indicates the parasite.

cells are still recognizable as erythroblasts, because of their nuclear structure. In Fig. 18, however, the parasite has reached its mature structure if not its mature size and the host cell nucleus is so distorted and changed in appearance that it can no longer be recognized as belonging to the red cell series. This is even more pronounced in the mature gametocytes shown in Figs. 19 and 20. It is these later stages that were first thought to be in the leucocytes, and it may indeed be true that some of the young forms do penetrate leucocytes. No such cases were seen, however, and many young parasites were examined in several of the birds.

The localization of immature stages of Leucocytozoon within the host. It is generally accepted that division in *Leucocytozoon* occurs in the internal organs, and some investigators have described the process. These dividing forms, however, are extremely difficult to find in natural infections. In the present series of natural infections the young stages, as illustrated in Figs. 14, 15, 16, and 17, were localized to a great extent in the bone marrow, and in one of the cedar waxwings by far the greatest number occurred in the brain.

INTRA-LEUCOCYTIC BODIES

In 20 of the finches and 7 *Molothrus* sp. oval or rounded bodies measuring from 5.4 micra to 6.3 micra long by 3.6 micra to 5.4 micra wide were found within what appeared to be monocytes. Dispersed, granular clumps of chromatin usually occupied a central position within these bodies (Figs. 5–13). From one to three such bodies were present in a single host cell, and they were frequently found in all of the internal organs examined, but never in the peripheral blood stream.

Aragão (1911, 1933), Marullaz (1913), Hoare (1924), Reichenow (1912), Carini and Maciel (1916), Nöller (1920), Herman (1937), Wood and Wood (1937), Manwell (1939), and Wolfson (1940) have described or pictured what appears to be this parasite from wild birds as well as from laboratory canaries known to be free from other blood parasites (Wolfson, 1940). A full description of the organism with detailed discussion as to its possible systemic position is given by Wolfson. It has been called *Toxoplasma, Haemogregarina,* and "Type II" toxoplasma-like bodies. It has undoubtedly also been confused with other exo-erythrocytic bodies thought by many contemporary workers to be part of the schizogonic cycle of malaria parasites. The organism resembles *Hepatozoon* in many respects although no schizogonic stages have ever been described. Due to the extreme uncertainty of its systemic position no attempt is made to give it a name.

Some of these parasites found in the present series of birds showed very definite structural relationships to the gametocytes of other intra-

corpuscular blood protozoa. In the figures shown in the plate, marked differences occur in the shape and distribution of the chromatin. For example, the chromatin of the parasite shown in Fig. 13 is far different from that shown in the other figures. Likewise the smaller stages of the parasite show different chromatin distribution than do the larger stages. A definite picture of development from the small forms to larger forms cannot be followed, however, and the stages of parasites cannot as yet be compared to the gametocytes of other intracellular protozoa until more is known about its life cycle.

Attempts were made to transmit this parasite to canaries by direct blood inoculation but all such attempts failed.

Trypanosoma

When the examination of dried and stained smears was first begun distorted masses of blue-staining cytoplasm with structures resembling kinetoplasts and undulating membranes were seen in the bone marrow, but these were at first not recognized as trypanosomes. Saline preparations made from the bone marrow, however, showed that many of the birds were positive for trypanosomes. Seventeen finches, 7 *Molothrus* sp. and 1 cedar waxwing harbored trypanosomes. They were not found in the peripheral blood or in any of the other internal organs even after extensive search. Minchin and Woodcock (1911), and Woodcock (1910) have noted a similar localization of bird trypanosomes in the case of *T. noctua* from the little owl and *T. fringillarum* of the chaffinch. They pointed out that trypanosomes were absent from the blood, but were found in the bone marrow, especially in winter and spring. In the present case the parasites were never numerous in the bone marrow and attempts to subinoculate them to canaries by injecting fresh saline suspensions of the bone marrow were not successful.

The morphological appearance in fresh and stained preparations of the trypanosomes found was similar to the descriptions which have been frequently made of other bird trypanosomes (Fig. 21). Small, thin forms were found as well as broad, flat organisms with heavily curled undulating membranes. In general structure they run the gamut of all the sizes and types which have been described from birds. Due to the fact that infections in canaries could not be obtained and their life cycle followed it is considered unwise to give them a specific name. Avian trypanosomes are remarkably polymorphic in structure and size measurements based upon organisms obtained from natural infections only are not dependable taxonomic material.

SUMMARY

1. Blood protozoa belonging to the genera *Plasmodium, Haemo-*

proteus, Leucocytozoon, and *Trypanosoma* are reported from three species of wild birds obtained in the vicinity of Mexico City. Sixty-one common house finches (*Carpodacus mexicanus frontalis*), 30 *Molothrus* sp. and 3 cedar waxwings (*Bombycilla cedrorum*) were examined.

2. Ten of the finches and 2 *Molothrus* sp. harbored plasmodia. Ten infections with *P. relictum* were found, one with *P. cathemerium,* and one with a small species resembling *P. nucleophilum.*

3. *Haemoproteus* was found in 13 of the finches.

4. *Leucocytozoon* occurred in all three species of birds, 7 infections being found in all. Young stages of this parasite were localized in the bone marrow and evidence is presented to show that these young stages occur in erythroblasts.

5. Intra-leucocytic parasites of uncertain systemic position were found in 20 of the finches and in 7 *Molothrus* sp. They could not be subinoculated to canaries.

6. Twenty-five birds, including all three species mentioned above, harbored trypanosomes in their bone marrow but not in any other part of the body examined. These were best found in fresh saline preparations of the marrow. Attempts to subinoculate them to canaries failed.

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A NEW SPECIES OF THE GENUS *CENTRORHYNCHUS* (ACANTHOCEPHALA) FROM THE BARRED OWL*

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The genus *Centrorhynchus* is poorly represented in the North American fauna. *Centrorhynchus spinosus* (Kaiser, 1893) and *C. californicus* Millzner, 1924, are the only species so far attributed to this continent. With an apparent wealth of members of this genus in South America, as revealed by the researches of Travassos, migratory birds seem to offer opportunity for introduction of forms from that continent, but there are no records of such dispersal in the literature.

In 1938, Dr. R. Harkema submitted to the senior author for identification specimens which he collected from a barred owl (*Strix varia varia*) taken in North Carolina. On cursory preliminary examination these seemed to conform to the general appearance of *C. spinosus*. A detailed study of these worms has revealed pronounced differences from any previously described species and hence they are recognized as belonging to a new species, which is here described as *Centrorhynchus conspectus*.

Centrorhynchus conspectus n. sp.

(Figs. 1 to 4)

Body very long, filiform; in mature females 28 to 55 mm long by 1 to 1.5 mm in maximum diameter, tapering very gradually to a diameter of 0.73 to 1.2 mm, posterior to which (Fig. 3) a swelling occurs, ranging from 1 to 1.5 mm in diameter. Beyond this swollen area the body tapers abruptly to about 0.49 mm at the extreme posterior tip. Mature males 23 to 35 mm long, lacking the conspicuous swelling (Fig. 4) near the posterior end. Proboscis (Figs. 1 and 2) usually 1.1 to 1.75 mm long though in one female it is fully extended and but 0.835 mm in length. The proboscis hooks are usually arranged in 26 to 28 longitudinal rows though in one instance 30 and in another 32 rows were observed. Each longitudinal row consists characteristically of 17 or 18 hooks with variability ranging from 16 to 19 as the extremes. These hooks show distinct differentiation (Fig. 2) as an anterior series of 4 or 5 large hooks with narrow, much elongated roots anterior to the line of insertion of the receptacle and a series of 12 to 15 smaller hooks posterior to the insertion. Hooks in the basal series of two distinctly different types. Those at base of proboscis are simple, thorn-like with very little root differentiation, while those on the anterior swollen region adjacent to the region of the receptacle insertion consist of two or three hooks with recurved cleft roots, some of which have conspicuous wing-like lateral projections extending anteriorly from the point where the hook unites with the root. The four or five large hooks at the anterior extremity of the proboscis vary considerably in length, most of them measuring 50 to 65 μ long, though in one female some of the anterior hooks were 73 μ long and in some males the largest hooks of this series were only about 44 μ

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long. The roots of these terminal hooks when viewed in surficial aspect are 52 to 88 μ long with an anterior neck region about 8 to 16 μ in diameter and the enlarged posterior widening of the root about 13 to 31 μ across. Hooks on enlarged portion of proboscis just posterior to insertion of receptacle wall are from 39 to 65 μ long with most of them between 50 and 60 μ. Thorns at base of proboscis range from 40 to 57 μ long with considerable variability in size.

The lemnisci (Fig. 1) are very long, often extending 1.5 mm beyond the posterior tip of the receptacle. The brain is located in the receptacle at about the level of the basal region of the proboscis.

The female genital orifice is distinctly subterminal. Fully embryonated eggs range from 60 to 78 μ by 21 to 26 μ with average size 68 by 21 μ.

In the male, the genital organs occupy about ¾ the length of the body. The testes (Fig. 1) may be slightly separated or in contact with each other. They are followed immediately by four extremely long cement glands, not all of which are always of the same length. In some instances one and in others two of the cement glands have their origin some distance posterior to the anteriormost of these glands.

Type host: Strix varia varia Barton (the barred owl), small intestine, January 4, 1938.

Type locality: Raleigh, Wake County, North Carolina.

Types: Holotype male (VC 3224.1), allotype female (VC 3224.14) and series of paratypes in the collection of H. J. Van Cleave, Urbana, Illinois.

The life history of *C. conspectus* is not known but for several species of this genus encysted larvae have been found in amphibians. Bent (1938: 189) records both frogs and salamanders in the list of foods of the barred owl. It is highly probable that some of these act as intermediate hosts though an arthropod is predicted as first larval host.

Comparisons: *Centrorhynchus conspectus* Van Cleave and Pratt falls into that group of species of this genus having 26 to 32 longitudinal rows of proboscis hooks and will be compared with the other members of this group. *C. conspectus* has much longer embryos than any other species in this series except *C. pinguis*. The proboscis of *C. pinguis* is 0.65 to 0.77 mm long which is only about one half the length in *C. conspectus*.

Key to the species of *Centrorhynchus* recognized from North American hosts

1 a) Proboscis armed with 48 longitudinal rows of 21 to 22 hooks each. *C. californicus* Millzner

b) Proboscis armed with not more than 32 longitudinal rows of hooks. 2

2 a) With 22 to 23 hooks in each longitudinal row of proboscis hooks. Hard shelled embryos 48 to 54 μ long. . . . . *C. spinosus* (Kaiser)

b) With 16 to 19 hooks in each longitudinal row of proboscis hooks. Hard shelled embryos 60 to 78 μ long. . . . . *C. conspectus* n. sp.

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EXPLANATION OF PLATE, P. 300

All figures were drawn with the aid of a camera lucida from stained whole mounts.

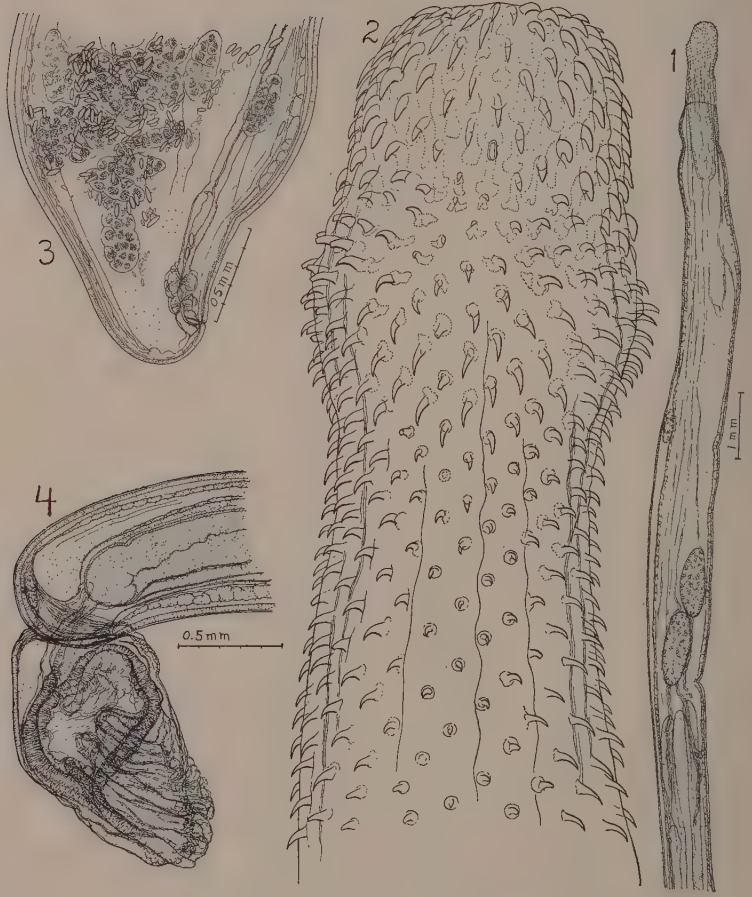
Centrorhynchus conspectus n.sp.

FIG. 1. Proboscis and anterior region of body of a paratype male showing relations of proboscis and receptacle and characteristic arrangement of the testes and cement glands. Note that three of the long, tubular cement glands are near the hind testis while the fourth does not extend so far forward.

FIG. 2. Proboscis of same individual as shown in Fig. 1.

FIG. 3. Posterior extremity of allotype female, showing genital tract and the characteristic swelling of the posterior region of the body.

FIG. 4. Posterior extremity of paratype male with characteristic bursa extruded.



Centrorhynchus conspectus n. sp.

FURTHER OBSERVATIONS UPON THE COMPOSITION OF ACANTHOCEPHALA*

THEODOR VON BRAND

In previous experiments (v. Brand, 1939 a) the chemical composition of the females of *Macracanthorhynchus hirudinaceus* has been studied to some extent. These studies have now been extended to the males of the same species and to some phases of worm biochemistry which so far have received no consideration. In addition, some new observations on the glycogen morphology of *Macracanthorhynchus* and two other Acanthocephala are given.

MATERIAL

Through the courtesy of Swift and Co., Chicago, it was possible to get long pieces of infected pig intestines a very short time after slaughtering. These pieces had been in contact with water for only a few minutes. The figures found for glycogen and other substances in the worms thus obtained were somewhat higher than those previously reported, probably because this time the contact of the worms with water was practically eliminated. As an additional precaution, only females and males attached at nearby places of the intestine were used for the comparative chemical determinations.

I am indebted to Dr. M. S. Ferguson for specimens of *Leptorhynchoides thecatus* from the sunfish, *Eupomotis gibbosus,* fixed with Carnoy's solution. *Echinorhynchus coregoni* was collected from infected perches, *Perca flavescens,* of Lake Michigan.

GENERAL CHEMICAL COMPOSITION OF FEMALES AND MALES OF *Macracanthorhynchus hirudinaceus*

The analytical methods were the same as those used previously (v. Brand, 1939 a). The results are summarized in Table 1.

Both females and males contained about the same amount of dry substance. The males, however, stored more glycogen and fat than the females. The values obtained in both sexes varied somewhat from experiment to experiment and this resulted in an overlapping of the extreme values. The glycogen figures for the males were consistently higher than those for the females in all ten determinations. The same was observed in five of the six ether extract determinations. The females yielded in one fat determination only a slightly higher value than

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TABLE 1.—*General chemical composition of females and males of* Macracanthorhynchus hirudinaceus

		Mean value and (extremes)	Total number of worms	Number of determinations
Weight of one ♀ in gm		4.1 (2.6– 6.0)	128	16
" " " ♂ " "		0.58 (0.39–0.95)	153	16
Dry substance in percent of fresh substance	♀	18.2 (12.8–20.8)	46	6
	♂	17.8 (12.7–22.8)	58	6
Glycogen in per cent of fresh substance	♀	1.35 (0.71–2.72)	82	10
	♂	2.27 (1.34–3.66)	95	10
Ether extract in per cent of fresh substance	♀	1.52 (1.12–1.80)	46	6
	♂	2.09 (1.62–2.70)	58	6

the males. It may be concluded from these observations that in *Macracanthorhynchus* there is a marked sexual dimorphism in respect to glycogen and also, though to a less extent, in respect to fat storage.

The figures for dry substance on the one hand and those for glycogen and fat on the other lead to the supposition that the males contain less protein than the females. Unfortunately no nitrogen determinations could be performed.

CHITIN IN *Macracanthorhynchus hirudinaceus*

During glycogen analyses according to Pflueger's method the worm bodies were dissolved with hot KOH of about 60 per cent. It was noticed that despite this treatment the innermost egg membrane remained intact and that in this way relatively large amounts could be collected. It is known that the eggshell proper of several nematodes consists of chitin (Fauré-Fremiet, 1913, Wottge, 1937, Chitwood, 1938); the alkali resistance noted above seemed to justify a search for the same substance.

The membranes isolated by heating with strong KOH and washed with distilled water were stained dark brown by iodine; upon addition of 1 per cent sulfuric acid the color turned dark blue, almost black. The membranes were insoluble in acetic acid of every strength, but soluble in concentrated hydrochloric and sulfuric acid. The substance could be reprecipitated from these solutions by neutralizing with alkali. After heating for two hours with concentrated hydrochloric acid in a boiling water bath the solution reduced Fehling's solution, probably because chitosamine had been formed.

The membranes withstood superheating with concentrated KOH at a temperature of 180° C for half an hour. After this treatment they

gave with iodine a pale violet color which turned purple after addition of 1 per cent sulfuric acid. They were now easily soluble in 3 per cent acetic acid, indicating that chitosan had been formed. After addition of N/10 sulfuric acid to such a solution, a precipitate, presumably of chitosan sulfate was formed.

There can be hardly a doubt that this egg membrane consists of chitin. It is another question, however, whether such chitin is identical with that found in arthropods. Some properties seem indeed to be slightly different. The fact that untreated egg membranes or membranes isolated by heating with weak alkali solutions gave no color reactions with iodine or iodine/sulfuric acid, may be explained on the assumption that the chitin is associated with substances preventing these color reactions, a fact occurring also in other cases. More curious is the observation that these membranes became soluble in 50 per cent acetic acid, although only slowly, if previously heated in a boiling water bath for six hours with strong KOH. In that case the reaction with iodine/sulfuric acid yielded a purple color. This would seem to indicate that under these conditions the transformation to chitosan had already begun. If heated, or especially if superheated, with strong alkali the membranes shrank greatly. They did not change their form, but merely shrank in size. The lumen finally represented a mere slit. From this observation, it would appear that the substance of this membrane has elastic properties. These are usually not present in normal chitin, but can probably be seen, at least to some extent, in freshly secreted chitin of other forms.

The amount of chitin in the eggs is fairly large. The sexual cells of several females were collected by centrifugation of the body fluid. After weighing they were heated for one half hour in a boiling water bath with 40 per cent KOH, washed twice (centrifugation) with distilled water, heated for two hours with 1 per cent HCl, washed twice with distilled water, heated for two hours with 60 per cent KOH, washed four times with distilled water, twice with hot alcohol 96 per cent and finally extracted for 24 hours with ether. After drying the substance was weighed. Sexual cells weighing 10.0 gm yielded 0.018 gm chitin, corresponding to 0.18 per cent. The eggs themselves must contain somewhat more chitin since by necessity mixed sexual cells (eggs and ovaries) had to be used in this determination.

The ability to secrete chitin distinguishes females from males. In the body of the latter no chitin could be found. The hooks of the proboscis are frequently referred to as chitinous. They were found to be indeed remarkably resistant to alkali. Even after superheating with alkali, it was sometimes possible to recover a hook of *Macracanthorhynchus* and *Echinorhynchus coregoni*. It had then, however, become granular and very brittle. It disintegrated entirely if touched lightly

with a needle. The hooks are therefore not quite as resistant against KOH as should be expected if they consisted of chitin.

No detailed study of the chemical composition of the remaining egg membranes was carried out, but some casual observations are recorded. The granular shell dissolved quickly in hot KOH, but seemed to be resistant against peptic and tryptic digestion. The middle membrane, which is everywhere in intimate contact with the chitin layer, swelled greatly under the influence of weak alkali. This layer is apparently responsible for the bursting open of the granular shell. If heated with strong KOH, this membrane dissolved. It can therefore not consist of chitin. In slides treated according to Bauer's procedure it became pink. This technique stains besides polysaccharides of the starch or glycogen type, also membrane carbohydrates like cellulose or tunicine. The possibility that a substance of that general type might be present should be remembered in future investigations. Meyer (1932/33, Fig. 369) calls the chitinous layer "Spaltprodukt" of the next membrane, his "Befruchtungsmembran" implying apparently that it is derived from the latter. The difference in chemical composition between the two layers is certainly not favorable to such a view.

MORPHOLOGICAL GLYCOGEN OBSERVATIONS

It has previously been stated (v. Brand, 1939 a and b) that the mature eggs of *Macracanthorhynchus hirudinaceus, Leptorhynchoides thecatus* and *Echinorhynchus coregoni* contain large polysaccharide masses in the region of the Embryonalkern of the embryo. In all three cases only a part of the substance appearing intensely red in sections stained with Ehrlich's haematoxylin—Best's carmin could be digested with saliva. This indicated either a pseudo-reaction, the presence of a polysaccharide resistant against salivary digestion or the association of glycogen with substances protecting it from digestion. It was especially surprising that the nuclei of the Embryonalkern retained Best's carmin with great tenacity, whereas the non-condensed nuclei in other regions of the embryo kept the blue haematoxylin color.

Further tests seemed called for. The nuclei of the Embryonalkern gave a very strong positive reaction for thymonucleic acid in slides stained according to Feulgen's technique. This shows definitely that we are dealing here with nuclei and not with accumulations of polysaccharide corpuscles comparable to paramylum bodies, as might have been inferred from the positive Best stain.

Other sections were stained according to Bauer's polysaccharide method and here the nuclei of the Embryonalkern remained entirely unstained. It is evident, therefore, that this part of the positive reactions in the Best slides was due to a pseudo-reaction. The Bauer slides

showed, in a quite similar way for the three species mentioned, the presence of small glycogen granules between the nuclei of the Embryonalkern, in a relatively dense zone just around it and to a lesser extent scattered through the protoplasm of the other parts of the embryo. Bauer's technique showed, in accordance with the previously reported findings, glycogen only in mature eggs.

A glycogen distribution essentially identical with that previously reported in *Macracanthorhynchus* was found in the bodies of *Lepthorhynchoides thecatus* and *Echinorhynchus coregoni*. Subcuticula, subcuticular lacunes and the protoplasmic parts of the muscles, both of the bodywall, the proboscis and the sexual apparatus of females and males were by far the most important storage places. A strong glycogen reaction was given by the muscular part of the receptaculum, but it was negative in the ganglion, contrary to what was found in *Macracanthorhynchus*. Relatively large amounts of glycogen were again found in the cement glands. It is still a matter of speculation whether it is used in the production of the secretion or whether it is an energy reserve for the gland cells. The secretion proper remained unstained also in slides stained according to Bauer's technique indicating that the polysaccharide underwent great changes if it was used in the elaboration of the secretion.

SUGAR IN THE BODY FLUID OF *Macracanthorhynchus hirudinaceus*

The polysaccharide found in ovaries and eggs undoubtedly originates from substances resorbed from the body fluid in which these cells are floating. Some glycogen occurs in the body fluid (v. Brand, 1939 a), but it seems unlikely that a polysaccharide could enter the cells directly. It was therefore decided to test the body fluid for carbohydrates of lower molecular weight.

In twelve experiments the reducing substances in the body fluid of females was determined according to Hagedorn and Jensen's technique. The mean value was 95 mgm per cent, the extremes were 44 and 165 mgm per cent. Many other substances besides sugar reduce ferricyanide. Therefore, in ten of the above twelve experiments parts of the body fluid were subjected to fermentation in order to decide how much of these reducing substances corresponded to fermentable sugar. The technique used was that employed in determining the rest reduction of blood. The results of these experiments are summarized in Table 2.

TABLE 2.—*Fermentable sugar and non-fermentable reducing substances in the body fluid of females of* Macracanthorhynchus hirudinaceus

	Fermentable sugar mgm per cent	Rest reduction mgm per cent
Mean value	51	34
Extremes	10 – 125	11 – 56

The amount of both fermentable sugar and non-fermentable reducing substances varied greatly from case to case. Some fermentable sugar, however, was present in every analyzed worm. The variations, which surpass in the case of the sugar by far those observed in other substances, make it probable that the nutritional stage of either the worm or the host is of great importance in determining the sugar level. It is definitely not as fixed as the blood sugar level for example in vertebrates.

AEROBIC GLYCOGEN CONSUMPTION OF *Macracanthorhynchus hirudinaceus*

The changes in chemical composition occurring in starving worms—the only way to gain definite knowledge about their type of energy production—is difficult to study. The chief reason is that they do not keep well in vitro. The worms were always sluggish, but after a few hours at 37° C in saline they became entirely motionless. This was not equivalent with death. If immersed for a short time in saline of 41° C they moved again sluggishly. In this way the worms were tested after the starvation period to ascertain whether they were living. In the individual experiment 9 to 10 female worms were analyzed at the beginning of the experiment and the same number after they had been kept for 20 to 23 hrs. at 37° C in saline in containers which had contact with air. Out of a greater series of experiments six only could be regarded as satisfactory, since in each of the others one or more worms died, making a glycogen determination impossible. The successful experiments gave the results summarized in Table 3.

TABLE 3.—*Glycogen consumption of females of* Macracanthorhynchus hirudinaceus *during starvation under aerobic condition at 37° C.*

	Glycogen content		Glycogen consumption of 100 gm worms in 24 hrs. in gm
	At beginning in per cent of fresh weight	After 20–23 hrs. in per cent of fresh weight at beginning	
Mean	1.16	0.65	0.8
Extremes	1.01 – 1.30	0.40 – 0.97	0.7 – 1.0

Weinland and Rudolph (1910) found in a single experiment conducted almost certainly under anaerobic conditions, a glycogen consumption of slightly less than 1.0 gm for 100 gm worms in 24 hrs. This would give a ratio between aerobic and anaerobic glycogen consumption of 1 : 1.2. This is very similar to that found in *Ascaris*, but different to that seen in free living worms or in a larval *Eustrongylides* (v. Brand 1938). This observation may be interpreted as meaning that in a way similar to *Ascaris, Macracanthorhynchus* has a prevalence of anaerobic processes also under aerobic conditions—at least in so far as processes involving glycogen are concerned.

I wish, however, to emphasize that both the figures given in this

paragraph and the conclusions drawn therefrom should be regarded as purely preliminary, owing to the uncertainties introduced by the low vitality of the worms in the starvation experiments. Further progress in this direction will be difficult, especially in so far as an isolation of metabolic endproducts is concerned. Contrary to what is seen usually in experiments with *Ascaris* or *Moniezia,* an enormous bacterial development takes place in the saline containing the acanthocephala. It will be difficult to attribute any substance isolated from the saline to the worms.

SUMMARY

1. The females of *Macracanthorhynchus* contain less glycogen and less fat than the males.

2. The innermost egg membrane consists of chitin.

3. No other structure containing chitin occurs in the acanthocephalan body.

4. An analysis of the Embryonalkern with staining methods shows that parts of the substances stained by Best's carmin are not glycogen.

5. Fermentable sugar and nonfermentable reducing substances were found regularly but in varying amounts in the body fluid.

6. Preliminary data on the glycogen consumption seem to indicate that the relations between aerobic and anaerobic processes having carbohydrate as mother substance are similar to those found in *Ascaris.*

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ENTONYSSUS VITZTHUMI (ACARINA), A NEW OPHIDIAN LUNG MITE*

FRED L. SCHMIDT

A large bull snake, *Pituophis sayi,* captured in Stillwater, Oklahoma, November 2, 1939, was found to harbor 32 specimens (6 male and 26 female) of an apparently new dermanyssid lung mite of the genus *Entonyssus* Ewing 1922.

The mites were fixed in hot Bouin's fluid and preserved in 70 per cent alcohol. Specimens cleared in Magath's glycerolactophenol and mounted in glycerine were used for temporary study; others were mounted in balsam.

Entonyssus vitzthumi n. sp.

(Figs. 1–12)

Features common to both sexes: Color milky white. Body proper (Figs. 4, 5) ellipsoidal, widest at level of legs III, about one and one-half times as long as wide, somewhat depressed.

Dorsal plate single, subelliptical, nearly covers dorsal surface; armed with a subsymmetrical pattern of basally articulate processes, including about 15 pairs of short stout spines of variable proportions distributed over the anterior two-thirds of the shield and a single pair of much longer slender setae on its posterior margin.

Capitula of typical form, continuously surrounded by a single chitinous shell having median forward dorsal and ventral extensions (the labrum and labium) enclosing the bases of the chelicerae. Front edge of labrum smoothly rounded. Front margin of labium (Fig. 8) with a median notch, each half being further divided into 3 lobes; medial lobes sharp-pointed, straight-sided, and hispid on anterolateral margins; middle lobes shorter, more blunt, smooth-edged, ventrad to and overlapping others; lateral lobes sharply pointed, smooth, and curved dorsad laterally along sides of chelicerae. Labium distinctly and abruptly raised (depressed in ventral view) in the area between the middle lobes and provided with 3 pairs of spines located one pair at the bases of the above-mentioned middle lobes and 2 pairs laterally near the base of the labium, somewhat in a transverse row—the members of the posterolateral pair being much the smallest of the group. Hypostome narrowly triangular with slightly concave sides, finely hirsute on both surfaces. Mouth not observed.

Legs (Fig. 1) nearly equal in length, IV being slightly the longest and I slightly the shortest. Legs II, III, and IV similar in shape and structure, II being somewhat the stoutest and IV the most slender. Spines present on all leg joints, somewhat variable in arrangement but with about 60 on each leg I, 50 on II, 40 on III, and 40 on IV; all spines of approximately the same shape and size except

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The writer is indebted to Professor George A. Moore of the same institution for the snake in which the new mite was found and to Mr. W. Eugene Hubbard for the information that he had found a few mutilated specimens of an undescribed mite in preserved bull snakes.

The new species is named in honor of H. Graf Vitzthum, Berlin, by whom *Entonyssus glasmacheri* was described.

that most of the coxal spines and 7 of the distal spines of tarsus I (Fig. 12) are more slender and much longer than the others. All tarsi provided with prominent strongly curved claws. Pulvillus (Fig. 10) of each foot cone-shaped with the base of the cone concave and distal and having its margin divided into 6 lobes, 4 of these (2 dorsal and 2 ventral between the claws) broad and short, the other 2 (located before and behind the others and adjacent to the claws) long and slender. The dorsal surface of the pulvillus is supported by 3 chitinous thickenings convergent at its base—2 of these are continued distally into the slender lobes, the third (middle) terminating near the notch between the dorsal lobes. All legs 7-jointed, pseudojoints being present in all femora and basal segments of tarsi, the pseudojoint of tarsus I very faint and only partially complete. Clawbars all prominent, each comprising 3 lobes, 2 symmetrically paired anteroposteriorly and one ventral; concavities between the middle and paired lobes enclose bases of claws.

Female: Body (Figs. 4 and 6) provided with widely separated dorsal, sternal, genitoventral, and anal plates, the intervening bare skin being finely wrinkled in a finger-print effect. Sternal plate longitudinally elongate, subhexagonal, situated at level of coxae II and III, bears 3 pairs of long spines one near each vertex of the hexagon. Genitoventral plate between coxae IV, unarmed, anteriorly truncate, posteriorly rounded, widest behind the middle. Anal plate subcircular; bears 3 long spines, located one posterior and 2 anterolateral to anus.

Chelicerae (Fig. 2) stout, pincer-like, with moderately chitinized basal sheaths. Fixed (dorsal) chela sharply bent near tip at an angle of slightly more than 90 degrees, nonalate; 2 characteristic tandem rounded serrations embrace the angle of curvature on its sharp edged ventral surface; a small mediodorsally situated spur at base of fixed chela armed with a single small spine. Movable chela falciform and provided with a single small ala on anterior half of its medial side. Fimbriate processes on anteroventral margin of socket housing movable chela.

Palpus (Fig. 11) apparently only 4-jointed, the terminal segment corresponding to the 2 distal palpal joints of the male and of either sex in other species; 31 spines distributed over the 4 joints, spines of 2 sizes, the longer situated near the distal end of the distal segment; one very stout furcate spine on medial side near middle of distal segment of palpus, spines more abundant on distal segment.

Male: Ventral plates united to form a single somewhat rectangular shield armed with 13 (1 median and 12 paired) long spines. Genital pore simple, in front portion of ventral plate.

Chelicerae (Fig. 3) modified, the fixed chela spurlike, supposedly functioning as an intromittent organ or "Spermatophorenträger" as described for *E. glasmacheri* by Vitzthum (1935). Movable chela bifurcate near middle into a long ventral pointed blade-like dorsally curved branch and a very short slender straight sharp-pointed dorsal branch closely appressed to the former, the line of bifurcation continued backward toward base of chela as a groove. Anteroventral margin of socket about base of movable chela fimbriated as in the female.

Palpus (Fig. 7) 5-jointed, its spination similar to that of female except that the furcate spine is lacking; its distal segment minute, 2-spined, and entirely ventrad to the penultimate segment.

Measurements: Averages in microns from 3 males or 4 females. L = length, W = width, D = diameter, F = female, M = male. Total L from anterior edge of labrum F 756 M 624. Trunk F L 708 W 372, M L 578 W 300. Dorsal shield F L 696 W 366, M L 570 W 294. F sternal plate L 187 W 66, genitoventral plate L 153 W 133, anal plate D 120. M ventral plate L 460. L of legs—F I 495 II 539 III 533 IV 594, M I 446 II 495 III 489 IV 537. L of tibiae—F I 60 II 66 III 55 IV 68, M I 57 II 60 III 45 IV 57. L of tarsi—F I 134 II 159 III 162 IV 192, M I 126 II 140 III 138 IV 159. L of distal segments of tarsi—F I 35 II 56 III 53 IV 50, M I 35 II 43 III 40 IV 39. Long D of claw bar F 16 M 14. Claws (M and F; P = L of point, B = L of base)—I P 14 B 11; II, III, and IV P 19 B 17. L of palpus M and F 100. L of 2 distal segments of chelicerae F 33 M 225.

Host: Pituophis sayi (Schlegel 1837) Cope 1898.

Habitat: Lung and trachea.
Locality: Stillwater, Oklahoma.
Cotype specimens: Two males and 4 females mounted in toto in balsam. In U. S. Nat. Mus.

The lung mites of snakes belong to 2 genera, *Entonyssus* Ewing 1922 and *Ophiopneumicola* Hubbard 1938, and include the following known species: (1) *O. bedfordi* (Radford 1937) mihi from *Dendraspis angusticeps* Smith; (2) *O. colubri* Hubbard 1938 from *Coluber flagellum flavi-gularis* (Hallowell); (3) *O. hamertoni* (Radford 1939) mihi from *Thamnophis sirtalis* (Linné); (4) *E. halli* Ewing 1922 from a "Pine snake"; (5) *E. rileyi* Ewing 1924 from a "Rattlesnake"; (6) *E. glasmacheri* Vitzthum 1935 from *Elaphe quadrivittata* (Holbrook); (7) *E. ewingi* Hubbard 1939 from *Crotalus cinereous* Le Conte in Hallowell; and (8) *E. vitzthumi* n. sp. from *Pituophis sayi* (Schlegel). *O. bedfordi* is a Rhodesian species; the others are all from North American hosts although the type specimens of *E. glasmacheri* and *O. hamertoni* were taken from snakes that died in captivity in Berlin and London respectively.

Radford's species, originally listed in *Entonyssus,* are transferred to *Ophiopneumicola* as indicated above because they more nearly conform to the diagnosis of the latter genus, particularly in not having the fixed chela of the female chelicera hooked or strongly recurved at the tip.

E. vitzthumi differs from *E. halli* (female) in that the chelae ("arm"s) do not conform to the description—". . . fixed arm harpoon-like, with a sharp point and a distal, backwardly directed barb, movable arm not extending to the tip of fixed arm . . .";

from *E. rileyi* (female) in that the fixed chela is not harpoon-like, the movable chela is not stouter than the fixed, the ultimate segment of the palpus apparently corresponds to both of the 2 distal segments in *E. rileyi,* the abdomen is much more elongate and not "naked," and the first leg is not one of the longer ones;

from *E. glasmacheri* in that the female fixed chela is not acutely recurved, no "Schultern" occur on the body or dorsal shield, no tritosternum is apparent, the female sternal and genitoventral plates are differently shaped and the latter is unarmed, the male ventral plate differently shaped, and the male movable chela is not "S-förmig";

from *E. ewingi* in the simpler form of the chelicerae, spiracles, hypostome, tarsi I, and labrum; different form of the labium; occurrence of only 4 distinct joints in the female palpus; presence of a single ventral plate in the male; and hair-like form of certain setae on the coxae and on most of the chitinous plates of the body.

For comparative study information was obtained from the original descriptions of the 7 previously known ophidian lung mites—the male of

E. ewingi having been first described by Hubbard (1940). In *O. colubri, E. halli,* and *E. rileyi* the males are as yet unknown.

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Explanation of Plate

All figures concern *Entonyssus vitzthumi* n. sp. and were made from adult specimens. Figs. 2, 3, 8, 10, and 12 are freehand studies; the others were drawn with the aid of a camera lucida, minor detail being supplied by freehand. In Figs. 1, 7, 11, and 12 the spines on the obverse and reverse surfaces are represented in solid and broken lines respectively.

Fig. 1. Female right legs, arranged in order I to IV, posterior views.

Fig. 2. Female left chelicera (2 distal segments), lateral view—note medial ala on movable chela. Double lines of chelae represent surfaces of solid chitin.

Fig. 3. Male left chelicera (2 distal segments), medial view. Stippled areas represent solid chitin.

Fig. 4. Female trunk, ventral view. Scale, 500 μ.

Fig. 5. Male trunk, ventral view—shows genital pore in front portion of ventral plate. Scale, 500 μ.

Fig. 6. Female trunk, dorsal view—shows spine pattern, anal seta, and spiracles. Scale, 500 μ.

Fig. 7. Male left palpus, ventral view. Scale, 50 μ.

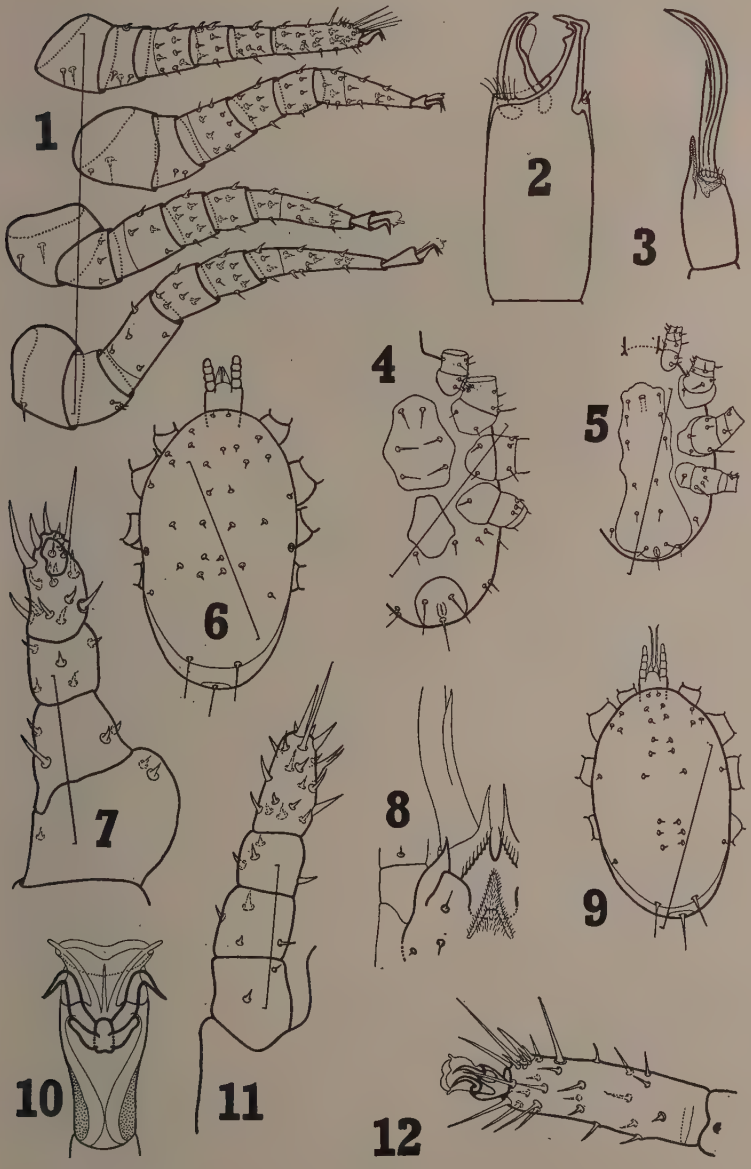
Fig. 8. Portion of male head, ventral view—note front margin of labium (heavy line) and hypostome. Along the transverse broken line the median anterior portion of the labium is abruptly elevated.

Fig. 9. Male trunk, dorsal view. Scale, 500 μ.

Fig. 10. Female left foot II, dorsal view—shows claws, clawbar, and pulvillus. Stippled areas represent solid chitin.

Fig. 11. Female right palpus, ventral view. Note medial furcate spine on terminal segment. Scale, 50 μ.

Fig. 12. Distal segment of female left tarsus I, ventral view—note the faint pseudojoint partially surrounding the proximal end.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NYCTOTHERUS BELTRANI N. SP., A CILIATE FROM AN IGUANA[1]

ROBERT HEGNER

The number of species belonging to the genus *Nyctotherus* (order HETEROTRICHIDA) is doubtful since several of those that have been reported from man and guinea-pig are probably not "good" species and the specific identity of others is questioned. *Nyctotherus* have been described from mammals, reptiles, amphibians, fish, snails, insects and other invertebrates. Among the reptiles, they have been reported from snakes, turtles and lizards. Grassé (1928) described a species (*N. haranti*) from a gecko (*Tarentola mauritanica*) in Tunisia. Only one of 400 geckos examined was infected. Johnston (1932) described another species (*N. trachysauria*) from *Trachysaurus rugosus* captured in southern Australia; the rectum of many lizards was inhabited with this species.

Of eleven iguanas (*Ctenosaura acanthura*[2]) captured near Cuautla, Morelos, Mexico, two contained in the cecum many trophozoites and a few cysts of a species for which I propose the species name *beltrani*, in honor of Dr. Enrique Beltrán, of the Instituto de Salubridad y Enfermedades Tropicales, who has contributed to our knowledge of *Nyctotherus* and has helped me in my investigations at this institute. The ciliates were not distributed uniformly throughout the cecum but were found in considerable numbers in some regions and were absent from others.

TROPHOZOITE

Shape and size. The body is oval in shape, the anterior portion being flattened and the posterior portion thicker. Measurements of 50 fixed and stained specimens gave a range in length of from 108 μ to 177 μ, the average length being 136 μ; and a range in breadth of from 64 μ to 135 μ, the average breadth being 93 μ. The largest of these 50 specimens measured 177 μ in length and 135 μ in breadth, and the smallest specimen measured 112 μ in length and 64 μ in breadth.

A peristomal groove extends from near the anterior end to the entrance of the cytopharynx. The cytopharynx enters the body obliquely and then bends posteriorly. Near the posterior end of the body is located a single contractile vacuole, and near this is a canal that opens to the outside through a cytopyge.

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[1] From the Department of Protozoology of the Instituto de Salubridad y Enfermedades Tropicales, Mexico, D. F.

[2] I am indebted to Prof. Rafael Martin del Campo for the identification of this species.

The length of the cilia is 6.5 μ and the distance between the rows of cilia is about 2.5 μ.

The macronucleus varies somewhat in shape, as indicated in the accompanying figures, and seems to vary in size according to the size of the specimen—the larger the specimen, the larger the macronucleus. Those of 50 trophozoites ranged from 26 μ to 85 μ in length and from 11 μ to 49 μ in breadth. The largest macronucleus measured was 85 μ long and 49 μ broad, and the smallest, 35 μ long and 11 μ broad. The average length was 52 μ and average breadth, 24 μ. The macronucleus is filled with spherules and granules of different sizes. Caryophore strands connect the macronucleus with the ectosarc as shown in Fig. 1. The small micronucleus lies close to one side of the macronucleus and is oval in shape.

Cyst

The cyst is also oval in shape with a thickening of the cyst wall at one end where there is a nipple-like protuberance. The average length of 17 cysts measured was 106 μ and average breadth, 69 μ. The range in length was from 94 μ to 117 μ and in breadth, from 59 μ to 85 μ. The largest of these cysts was 117 μ long by 85 μ broad, and the smallest was 95 μ long and 59 μ broad.

The macronucleus lies near the posterior end of the cyst and is oval in shape. The average length of 17 nuclei measured was 28 μ and the average breadth, 21 μ; thus the nucleus of the cyst is much smaller than that of the trophozoite. The range in length of the macronucleus was from 23 μ to 33 μ and in breadth, from 17 μ to 26 μ. The largest macronucleus measured was 32 μ long and 26 μ broad; the smallest was 23 μ long and 17 μ broad. Between the macronucleus and the posterior end of the cyst was a group of glycogen-like bodies.

Nyctotherus beltrani n. sp.

Trophozoite. Length: 108 μ to 177 μ, average, 136 μ. Breadth: 64 μ to 135 μ, average, 93 μ. Shape: broadly oval. Cytopharynx enters body obliquely and then bends posteriorly. One contractile vacuole near posterior end. Anal canal opens through cytopyge near posterior end. Cilia 6.5 μ in length in parallel rows 2.5 μ apart. Macronucleus: elongated and variable in breadth; 26 μ to 85 μ long, average, 52 μ; 11 μ to 49 μ broad, average, 24 μ; caryophore strands present. Micronucleus partly embedded in macronucleus.

Cyst. Oval in shape with nipple-like protuberance at anterior end. Length: 94 μ to 117 μ, average, 106 μ. Breadth: 59 μ to 85 μ, average, 69 μ. Macronucleus oval in shape; 23 μ to 33 μ long, average, 28 μ, and 17 μ to 26 μ broad, average, 21 μ. Between macronucleus and posterior end, a group of glycogen-like bodies.

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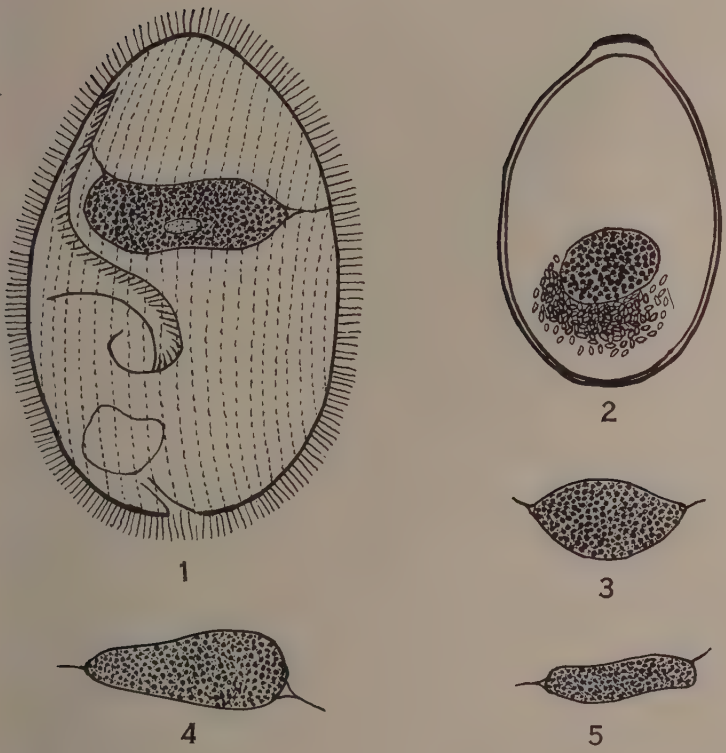

FIG. 1. Trophozoite of *Nyctotherus beltrani*, n. sp. from an iguana. (×500.)
FIG. 2. Cyst. (×500.)
FIGS. 3, 4, 5. Nuclei from trophozoites showing variations in shape and size. (×500.)

A NEW GENUS AND NEW SPECIES OF AMOEBAE FROM MEXICAN LIZARDS*

ROBERT HEGNER and REDGINAL HEWITT

Intestinal amoebae have been reported from several types of reptiles,—turtles, lizards and snakes. While in Mexico during the summer of 1939, the writers had an opportunity to examine the intestinal contents of three species of lizards in which they found the trophozoites and cysts of endamoebae that appear to be new species. In addition, spherical bodies of unknown character were observed that resembled the cysts of endamoebae. Twenty-nine small lizards (Iguanidae) of the species *Sceloporus clarkii* were examined; in 11 of them spherical cyst-like bodies were seen. Of 11 large iguanas, *Ctenosaura acanthura,* 4 were positive for coli-like endamoebas, 2 were positive for an endamoeba characterized by a nucleus with a wrinkled membrane, one was positive for a histolytica-like endamoeba, and 3 were positive for spherical cyst-like bodies. One horned toad (*Phrynosoma* sp.) also contained spherical cyst-like bodies.

1. *Endamoeba ctenosaurae* n. sp. (Figs. 1 to 7). Four of 11 iguanas (*Ctenosaura acanthura*) were positive for this coli-like species. Twenty trophozoites were measured. They ranged in length from 17 μ to 34 μ (average, 22 μ) and in breadth, from 10 μ to 30 μ (average, 17 μ). The nucleus of the trophozoite ranged in diameter from 4 μ to 8 μ (average, 5.6 μ). Figs. 1 and 3 represent typical trophozoites, and Figs. 4 to 7, variations in the structure of the nuclei.

The cysts of this species did not contain chromatoid bodies; their wall was frequently "beaded." The range in diameter of 25 cysts measured was 15 μ to 23 μ (average, 19 μ). Cysts were observed with from 1 to 16 nuclei, but the most frequent numbers were 2 or 8. The nuclei ranged in diameter from 2 μ to 6 μ (average, 3.6 μ).

This new species is named after the genus of iguanas in which it was discovered.

2. *Martinezia baezi* n. g., n. sp. (Figs. 8, 9, 12–14). Two iguanas (*Ctenosaura acanthura*) were infected with an amoeba which we believe belongs to a new genus. No cysts were found but the nucleus of the trophozoite differs from that described for other genera. It contains a large karyosome that may or may not be granular in appearance. A heavy layer of chromatin, either homogeneous or in the form of beads, is located on the nuclear membrane. The nuclear membrane is usually wrinkled;

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* From the Instituto de Salubridad y Enfermedades Tropicales, Mexico, D. F. We are indebted to Drs. Beltrán and Aquilar for assistance in obtaining material and the correct scientific names of the lizards.

this is not due to poor fixation since the nuclei of the coli-like amoebae (Figs. 1–7) that were present on the same slides were well fixed. Three per cent of the trophozoites contained two nuclei each. The length of 100 trophozoites ranged from 8 μ to 21 μ (average, 13 μ) and the breadth ranged from 6.5 μ to 16 μ (average, 10 μ). The nucleus of 100 trophozoites ranged in diameter from 3 μ to 5 μ (average, 4 μ).

We have named this species in honor of Dr. M. Martínez-Báez who was director of the Instituto de Salubridad y Enfermedades Tropicales, Mexico, D.F., when this work was done and who helped us in every possible way in our studies.

3. *Endamoeba cuautlae* n. sp. (Fig. 11). One iguana (*Ctenosaura acanthura*) was infected with histolytica-like amoebae. Many cysts were observed but no trophozoites that could be determined definitely as belonging to this species. The cysts were spherical in shape and the protoplasm in most of them was pulled away from the cyst wall as shown in Fig. 10. This was not due to poor fixation because no shrinkage was observed in the cysts of coli-like amoebae (see Fig. 2) on the same slides. The cysts contained characteristically four nuclei of the histolytica type. From 1 to 6 rod-shaped chromatoid bodies were present, either singly or in pairs. Forty cysts ranged in diameter from 8 μ to 14 μ (average, 11 μ). The nuclei ranged in diameter from 1.3 μ to 4 μ (average, 2.2 μ). We have named this species after the city (Cuautla, Morelos) near which the infected iguana was captured.

4. Spherical cyst-like bodies (Fig. 11) were noted in the intestinal contents of 11 of 29 specimens of *Sceloporus clarkii,* in 3 of 11 specimens of *Ctenosaura acanthura* and in one horned toad (*Phrynosoma* sp.). The cytoplasm in these "cysts" was definitely reticulate. The large nucleus was spherical and contained a large, spherical karyosome, which was often solid but frequently appeared to contain several large granules embedded in a lighter-staining matrix, resembling in this respect the nucleus of *Dientamoeba.* No chromatin was located on the nuclear membrane. Linin fibers were visible in many of the nuclei between the karyosome and the nuclear membrane. The range in diameter of 59 cysts was 7 μ to 14 μ (average, 9 μ), and of the nuclei, 2 μ to 3.6 μ (average, 2.5 μ). Cysts of this type were found throughout the digestive tract of the horned toad from the stomach to the rectum. Their nature is not known to us.

SUMMARY

One new genus, and three new species of amoebae are described from lizards collected in Mexico, D.F., and near Cuautla, Morelos. Cyst-like bodies of unknown nature are also described.

FIGS. 1–7. *Endamoeba ctenosaurae* n. sp. FIGS. 1 and 3. Trophozoites. FIG. 2. Cyst containing 8 nuclei. FIGS. 4–7. Variations in the structure of the nucleus.

FIGS. 8–9, 12–14. *Martinezia baezi* n. g., n. sp. FIGS. 8–9. Trophozoites. FIGS. 12–14. Variations in the appearance of the nuclei.

FIG. 10. *Endamoeba cuautlae* n. sp. Quadrinucleate cyst of a histolytica-like amoeba from an iguana captured near Cuautla, Morelos.

FIG. 11. Drawing of a cyst-like body encountered in three species of Mexican lizards.

EFFECT OF TROPICAL SUNLIGHT ON EGGS OF *ASCARIS SUIS* (NEMATODA), THE LARGE INTESTINAL ROUNDWORM OF SWINE[1,2]

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Infections with large intestinal roundworms (*Ascaris suis*) constitute a problem in swine production that is of considerable economic importance. These worms are among the most widespread and injurious of the intestinal helminth parasites that infect pigs (Ransom, 1927, Burch, 1930, Roberts, 1934). Light infections may produce some degree of unthriftiness and retarded growth while severe infections may result in death of the affected animal (Schwartz, 1937).

The widespread distribution of ascarids among pigs is attested by the high incidence of infection reported whenever the occurrence of this parasite in swine has been investigated. In this connection, the writer (Spindler, 1934) examined at autopsy 349 swine of all ages originating in Georgia, Florida, and Alabama, and found 74 per cent to be infected with ascarids. In Australia, Roberts (1934) examined 46,433 swine of all ages originating in Queensland and reported that 20 per cent harbored ascarids. On the other hand, available information indicates that these nematodes may be somewhat less prevalent in some tropical regions. In this connection, Schwartz (1922) reported them to be comparatively rare in adult swine examined by him in the Philippine Islands. Payne, Ackert, and Hartman (1925) found only 8.8 to 10.8 per cent infection in pigs they examined in Trinidad. The present writer observed 19 infections in 100 hogs of all ages examined in the municipal abattoir in Mayaguez, Puerto Rico, during May, June, and July, 1936; the infections were for the most part very light (unpublished data).

The apparent low incidence of ascarids in swine in some portions of the tropics compared to their prevalence in most temperate regions suggests the existence in the tropics of some factors, other than animal husbandry practices, that may operate to keep at a low level the ascaris burden of swine. These factors, some of which are probably environmen-

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[1] These investigations were carried out from May 14 to August 6, 1936, at the U. S. Department of Agriculture Experiment Station at Mayaguez, and at the School of Tropical Medicine and Hygiene, San Juan, Puerto Rico.

[2] The use of *Ascaris suis* in this paper is not intended to imply that the author considers the ascarids that parasitize swine to constitute a morphological entity; it is used for convenience and to insure that the reader understands that in all cases ascarids of swine are referred to.

tal, are apparently less effective under conditions existing in many portions of temperate regions where hogs are raised on a large scale.

Intense sunlight is generally considered to be instrumental in hastening the death of parasite eggs and larvae on soil under natural conditions. However, the effectiveness of sunlight, apart from the thermal and drying action, in destroying the vitality of eggs of *Ascaris suis* has to a certain extent been a matter of conjecture. The limited series of investigations heretofore carried out failed to separate effectively the various factors involved. For example, Roberts (1934) reported that fresh eggs of *A. suis* exposed in water to sunlight were killed in from 4 to 6 hours; dried fresh eggs did not live more than 2 hours. Embryonated eggs in water were killed by periods of exposure ranging from 3 to 4 hours; embryonated eggs in a dried condition were killed in 1 to 2 hours. However, Roberts observed that the temperatures of the medium surrounding the eggs frequently rose above 40° C during the period of exposure and later he found that temperatures of 40° C or higher, in the absence of sunlight, effectively inhibited the development of eggs.

In view of the fact that little definite information was available concerning the effect of intense sunlight on eggs of *Ascaris suis,* 14 tests were carried out in which ascarid eggs in different stages of development were exposed to direct sunlight for varying periods, in water and in the dry state. It was considered that precise information of the effect of sunlight on these eggs would not only aid in explaining the low incidence of ascarids in some tropical regions but that it would also extend existing knowledge of factors that influence the spread of this and other parasites of livestock under tropical conditions. It was considered also that information of this type might explain the comparatively high incidence of ascarids in temperate regions and the information elicited could, perhaps, be used as a basis for improving control measures for roundworms and other parasites of swine.

The tests discussed in this paper have already in part been briefly reported (Spindler, 1936 a and b).

EXPERIMENTAL PROCEDURE

Source of eggs.—Eggs used in tests herein described were removed from the uteri of gravid female ascarids obtained from infected swine post mortem. In order to free the masses of eggs from uterine tissue, the chopped-up uteri together with the eggs were placed on a screen having 16 meshes to the inch and the eggs were forced through the meshes by alternate rubbing and washing. The eggs concentrated in tap water in stoppered flasks were then washed by vigorously shaking the flasks, allowing the eggs to settle and discarding the supernatant fluid. This was repeated until the eggs were freed of most of the worm tissue. Various degrees of

development in eggs for different tests were obtained by culturing them in shallow tap water in the laboratory until they attained the stage of development desired.

Several mosquito larvae were kept in each stock and in each test and control culture. The larvae by their almost constant activity kept the eggs stirred and aerated and this prevented them from clumping and sticking to the dishes. These larvae also consumed molds and other vegetable growths which might have become so thick in the cultures as to retard development of the eggs. It was observed that the mosquito larvae occasionally ingested eggs and later voided them with fecal material. Passage through the alimentary canal of the mosquito larva apparently did not injure the eggs so far as their ability to develop was concerned. As the mosquito larvae became ready to metamorphose into adults, they were removed from the cultures, destroyed and replaced by young larvae that had hatched in tap water.

Four stages in the development of the ascaris eggs were tested; these stages were designated as follows: undeveloped (one cell); early segmentation (2 to 32 cells); late segmentation, embryonated (embryos fully formed and active).

Description of tests.—In 9 tests, several cultures, each consisting of approximately 5,000 eggs in Syracuse watch glasses of water (temperature 30° C) were exposed to direct sunlight for varying periods (Table 1). In tests 1, 2, and 4, undeveloped eggs were used; in tests 6 and 7 eggs in late segmentation stages were exposed; in tests 3, 5, 8, and 9 embryonated eggs were used.

To ascertain the effect of sunlight combined with drying, but apart from the heat, cultures of approximately 5,000 eggs each were dried in Syracuse watch glasses and then exposed to direct sunlight in 5 tests (10 to 14, Table 2). Test 10 involved undeveloped eggs; in test 14 eggs in early segmentation stages were used; test 13 involved eggs that were in late segmentation stages; in tests 11 and 12 embryonated eggs were exposed.

Technique of exposing eggs to sunlight.—The watch glasses containing cultures of eggs (either in water or in a dried condition) were placed in crystallizing dishes each containing water (temperature 30° C) deep enough to come well up on the sides of the watch glasses but not deep enough to overflow the edges; each crystallizing dish was then placed on a white metal background in direct sunlight. At frequent intervals during exposure of the eggs water was siphoned off from the crystallizing dishes and immediately replaced with water having a temperature of 30° C. This process was repeated often enough to maintain the temperature of the water in the crystallizing dishes between 30 and 35° C.

At intervals (see tables) individual cultures were removed from the

TABLE 1.—*Summary of data on the effect of tropical sunlight on eggs of* Ascaris suis *in shallow water**

Test number	Date 1936	Culture number	Designation of stage of development	Eggs in cultures exposed to sunlight				Eggs in control (unexposed) cultures
				Time exposed	Duration of exposure (hours)	Date examined 1936	Per cent dead	Per cent dead
1	May 29	1	Undeveloped	12:55 PM to 1:25 PM	½	June 17	8	
		2	"	" to 1:40 PM	¾	"	9	5
		3	"	" to 1:55 PM	1	"	15	
2	June 6	1	"	1:00 PM to 4:00 PM	3	June 29	100	5
		2	"	" to 4:30 PM	3½	"	100	
3	June 18	1	Embryonated	10:00 AM to 12 noon	2	July 8	21	
		2	"	" to 1:00 PM	3	"	21	
		3	"	" to 1:30 PM	3½	"	44	5
		4	"	" to 2:00 PM	4	"	62	
		5	"	" to 3:00 PM	5	"	90	
		6	"	" to 3:30 PM	5½	"	100	
4	June 19	1	Undeveloped	9:00 AM to 12 noon	3	July 9	100	4
		2	"	" to 12:30 PM	3½	"	100	
5	June 19	1	Embryonated	8:00 AM to 2:00 PM	6	July 9	100	5
		2	"	" to 2:30 PM	6½	"	100	
		3	"	" to 4:00 PM	8	"	100	
		4	"	" to 5:00 PM	9	July 10	99.8	
6	June 22	1	Late segmentation	8:00 AM to 10:00 AM	2	July 10	16	
		2	"	" to 11:00 AM	3	"	26	
		3	"	" to 11:30 AM	3½	July 11	56	3
		4	"	" to 12 noon	4	"	70	
		5	"	" to 12:30 PM	4½	"	100	
		6	"	" to "	4½	"	100	
7	June 27	1	Late segmentation	8:00 AM to 12:30 PM	4½	July 15	100	2
8	July 7	1	Embryonated	8:00 AM to 1:00 PM	5	July 27	92	
		2	"	" to 1:30 PM	5½	"	100	4
		3	"	" to 2:00 PM	6	July 28	100	
		4	"	" to 4:00 PM	8	July 29	100	
		5	"	" to 5:00 PM	9	July 30	100	
9	July 28	1	Embryonated	8:00 AM to 4:00 PM	8	August 17	100	7
		2	"	" to 5:00 PM	9	"	100	

* A total of 3,000 eggs examined from each culture; control cultures examined the same day as the respective exposed cultures; in tests 5, 6, and 8 on the day of the last exposed cultures.

TABLE 2.—*Summary of data on the effect of tropical sunlight on eggs of* Ascaris suis *in a dried condition**

Test number	Date 1936	Culture number	Designation of stage of development	Eggs in cultures exposed to sunlight				Eggs in control (exposed) cultures
				Time exposed	Duration of exposure (hours)	Date examined 1936	Per cent dead	Per cent dead
10	June 18	1	Undeveloped	9:00 AM to 11:10 AM	2⅛	July 6	100	3
		2	"	" to 11:45 AM	2¾	"	100	
11	June 24	1	Embryonated	10:00 AM to 10:30 AM	½	July 14	14	
		2	"	" to 11:15 AM	1¼	"	53	
		3	"	" to 11:30 AM	1½	July 15	57	4
		4	"	" to 11:45 AM	1¾	"	67	
12	June 30	1	Embryonated	8:00 AM to 12:30 PM	4½	July 20	99	
		2	"	" to 1:00 PM	5	"	100	
		3	"	" to 1:30 PM	5½	"	100	1
		4	"	" to 2:00 PM	6	July 21	100	
		5	"	" to 5:00 PM	9	"	100	
		6	"	" to " PM	9	"	100	
13	July 1	1	Late segmentation	8:00 AM to 12 noon	4	July 21	93	3
		2	"	" to 12:30 PM	4½	"	100	
		3	"	" to 1:00 PM	5	July 22	100	
14	July 28	1	Early segmentation	8:00 AM to 9:30 AM	1½	August 15	99	
		2	"	" to 10:00 AM	2	"	100	5
		3	"	" to 12 noon	4	"	100	
		4	"	" to 1:00 PM	5	"	100	
		5	"	" to 1:30 PM	5½	"	100	
		6	"	" to 2:00 PM	6	"	100	

* A total of 3,000 eggs examined from each culture; control cultures examined the same day as the respective exposed cultures; in tests 11, 12, and 13 this was July 15, 21, and 22; in test 14 the control was examined August 17.

dishes, promptly covered to exclude sunlight and removed to the laboratory. In the case of eggs in water, the water was replaced with fresh tap water (temperature 30° C); in the case of dried eggs, water was added to the dishes.

In each test a control culture of approximately 5,000 eggs, either in water or dried to correspond to the condition of the test cultures, was subjected to a temperature of 30 to 35° C simultaneously with and during the longest period test cultures were exposed to sunlight. Eggs in the control cultures were always of the same stage of development as eggs in the corresponding test cultures and were at no time exposed to sunlight.

The measure of the effect of sunlight on the vitality of the eggs was a comparison of the ratios of live to dead eggs in various exposed cultures with those in unexposed (control) cultures. In order to ascertain this the cultures were first kept 18 to 20 days in the laboratory to allow time for degenerative changes to become apparent in dead eggs and to permit those not dead to recover from the effects of exposure and begin development. At completion of these culture periods 6 representative samples of 500 eggs each (total, 3,000 eggs) from each test and each control culture were examined microscopically to ascertain the proportion of live to dead eggs. The criteria used to determine whether eggs were alive or dead were as follows:

In the case of eggs that were incompletely developed (not embryonated) at the time of exposure, those that failed to develop further and which on microscopic examination showed granulation, vacuolation or clearing of the protoplasm were considered dead. In the case of embryonated eggs, embryos that were vacuolated, and the protoplasm granular in appearance and which failed to become active when subjected to a temperature of approximately 37° C were considered dead.

EXPERIMENTAL RESULTS

Results of tests to ascertain the effect of tropical sunlight apart from heat on eggs of *Ascaris suis* in shallow water and dried on glass are summarized in the accompanying tables. The following brief presentation of the data will serve to point out some of the more outstanding facts elicited.

Eggs exposed in shallow water.—From a consideration of the data presented in Table 1, it can be seen that whereas eggs in all stages of development failed to survive comparatively short exposures to direct sunlight, eggs in advanced stages of development were noticeably more resistant to its inimical effects than were undeveloped (one-celled) eggs. As shown in Table 1, 85 per cent of the undeveloped (one-celled eggs, test 1) survived an exposure of 1 hour; none survived as long as 3 or more hours in direct sunlight (tests 2 and 4). On the other hand, 30 per cent of eggs in the late segmentation stages survived 4 hours (test 6). However, the

rate of death of eggs in cultures exposed longer than 4 hours was apparently very rapid as no eggs survived 4½ hours (Table 1).

Embryonated eggs were apparently more resistant to sunlight than either undeveloped or developing eggs. This is shown by the fact that in test 5 a small proportion (0.2 per cent of those counted) remained alive even after continuous exposure for as long as 9 hours. However, in 2 subsequent tests none of the embryos contained in eggs exposed 5½ hours or longer, survived (tests 8 and 9 in Table 1).

That the death of the vast majority of eggs in the cultures exposed to sunlight can be ascribed to its inimical effects is attested by the fact that 93 to 98 per cent of eggs examined from the various control cultures were alive at the completion of the tests (Table 1).

Eggs exposed in a dried condition.—The results of 5 tests involving eggs in a dried condition exposed to sunlight are summarized in Table 2. In the case of undeveloped eggs, 2 1/6 hours was the shortest period any eggs were exposed (test 10); none of these eggs survived. In the case of eggs in the early segmentation stages, none survived longer than 1½ hours (test 14). Eggs in the late segmentation stages (test 13) were somewhat more resistant, as a small proportion (7 per cent) was able to continue development following 4 hours exposure, however, none survived periods of exposure as long as 4½ hours. It is interesting to note in this connection that dried embryonated eggs were apparently only slightly more resistant than were eggs in the late segmentation stages similarly exposed in test 13; no dried embryonated eggs survived periods of exposure of 5 hours or more (test 12).

In the case of eggs in control cultures from 95 to 99 per cent of those examined at completion of the tests were alive (Table 2). This fact demonstrates that death of the vast majority of eggs in the exposed cultures was due to the inimical effects of sunlight combined with desiccation.

DISCUSSION

In tests carried out and reported in this paper, eggs of *Ascaris suis* both in shallow water and in a dried condition were killed by short continuous exposures to sunlight when the temperature of the medium surrounding the eggs, in so far as could be ascertained, varied between 30° C and 35° C; similar temperatures exerted no appreciable deleterious effect on eggs in control cultures.

It was observed during the course of these tests that eggs in cultures exposed to sunlight did not all die as a result of the same period of exposure, but the number of dead eggs in the cultures increased rapidly as the exposures were lengthened. In general, small numbers of eggs were able to survive exposures that destroyed the vitality of the majority of eggs in the cultures. A similar phenomenon was observed by Wright

(1936) in connection with tests of the effect of ultraviolet light on eggs of *Toxascaris leonina* and *Toxocara canis*. Wright found that while the vast majority of eggs in cultures exposed were killed, apparently by the "dosage" of ultraviolet light used, a few eggs were able to survive and complete development.

It was further observed during the course of tests under consideration that somewhat longer periods of exposure were generally required to bring about the death of fully developed eggs than was required in the case of undeveloped eggs. This is in agreement with the findings of Shalimov (1935), who reported that the embryonated eggs of horse ascarids (*Parascaris equorum*) are markedly more resistant to the inimical effects of ultraviolet light than the undeveloped eggs.

Under natural conditions ascarid eggs would be more or less protected by the fecal mass during early stages of development. This, together with the heightened resistance to solar radiation later in development, probably accounts for long survival of some ascarid eggs even under tropical conditions.

SUMMARY

Fourteen tests designed to ascertain the effect of tropical sunlight (apart from its heat) on eggs of the swine ascarid were carried out under conditions existing in Puerto Rico. In 9 tests eggs in shallow water were exposed to sunlight; in five other tests eggs were first dried in glass dishes and then exposed.

Undeveloped eggs both in water and dried were less resistant to the effects of sunlight than fully developed eggs. All the one-celled eggs in water failed to survive continuous exposure to sunlight as long as 3 hours, and none survived 2¾ hours exposure in a dried condition; some embryonated eggs in water survived 5 hours, and some as long as 9 hours. Embryonated eggs exposed in a dried condition failed to survive exposure periods as long as 5 hours.

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RESEARCH NOTES

NOTE ON THE OCCURRENCE OF MICROSPORIDIAN INFECTION AMONG NATIVE SCARABAEID LARVAE

In the examination of field plots at Cape Charles, Va., where studies were under way on the diseases of the Japanese beetle (*Popillia japonica* Newm.), several larvae of *Strigodermella pygmaea* (F.) were found on October 11, 1938, which showed infection of the fat bodies with a microsporidian. In another survey of the area on November 1 and 2, 15 out of a total of 250 third-stage larvae of this species were found similarly affected, and in addition 2 out of 14 larvae of *Phyllophaga gracilis* (Burm.) were also found to be infected by a microsporidian. The microsporidia involved seemed to be identical for the two species.

The spore forms of the microsporidian are ovoid refringent cells, with a clear rounded vacuole in the broader pole. The spores measure 5.3 to 5.8 microns in length and 3.2 to 3.9 microns in breadth. The polar filament is extruded from the narrow pole of the spore and is long and slender, measuring over 50 microns in length. Extrusion is accomplished with difficulty by direct mechanical pressure, but is easily effected by introducing a small amount of 5% sodium hypochlorite at one side of the cover slip. When the reagent penetrates under the slip and reaches the spore, there is a marked disturbance in the membrane at the narrow pole and at once the filament is extruded with explosive violence. After the extrusion of the filament, the spore shows a pyriform vacuole which occupies almost the entire cell. In the fresh state the spores stain readily with aqueous methyl violet and similar dyes. They take up iodine readily, staining a deep brownish yellow. The spores do not stain deeply with methylene blue or Wright's stain.

Attempts to produce artificial infection in larvae of the two species in which infection was found naturally and in *Popillia japonica* have not yet been successful. —S. R. Dutky and R. T. White, *Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.*

PERMANENT MOUNTS FROM NIH SWABS POSITIVE FOR PINWORM OVA

The NIH (National Institute of Health) swab devised by the late Dr. Maurice C. Hall for the diagnosis of pinworm infection has proven a highly efficient tool in our hands during the course of a recent hospital pediatrics survey, and subsequently in routine laboratory studies. The cellophane perianal scraper squares, easily prepared, easily employed and easily examined with a very high surety of diagnosis, have here supplanted all other methods for ascertaining infections with *Enterobius vermicularis.*

In cases where positive NIH swabs were very heavy in egg load, it was desirable to attempt to preserve them for class study and teaching use, since we realized the force of illustration with actual clinical materials. We have considered glycerine jelly so far to be the most satisfactory mounting and preserving medium, since routine NIH swab square examinations are carried out by flattening the cellophane in water or saline solution before examining by microscope. Running a square loaded with ova through an alcohol-xylene series for dehydration and balsam mounting causes the loss of so high a percentage of the eggs that we have thought the aqueous medium of glycerine jelly to be technically more feasible. Our jelly has been a 0.5% phenolic glycerine jelly (Coleman and Bell Co.), but without doubt the several other types of this jelly (arsenious acid, thymol, etc.) would be equally serviceable.

The cellophane square, following preliminary examination in physiological saline solution to confirm the presence of pinworm ova, was lifted by forceps, and inverted in a drop of jelly upon a slide. To flatten the wrinkled cellophane, and to seal the preparation, a second drop of jelly was placed upon the upper surface of the cellophane, and a thin glass coverslip of suitable size inverted upon it. We have further sealed our preparations with model airplane glue, finding it a tough, easily handled material superior in our hands to gold size or balsam for ringing such mounts; its

longevity as yet we cannot indicate. Swabbings stained with colloidal iodine (Chandler), to kill the embryos and to bring out the egg shell for easier quick identification, rapidly lost all color, but without destruction of the egg outline, or changes in the embryo contained within. To this date, one year, the ova, stained or unstained, have all retained both general conformation and internal structure.

Glycerine jelly mounts are, of course, relatively very fragile. In laboratory sets, with the customary careless student handling, glycerine jelly slides suffer a high breakage mortality. Moreover, either sealed with gold size, or left unsealed (arsenious acid jelly), there is tendency for evaporation of the bound water in the jelly; shrinkage of the jelly occurs, and ultimately loss of the specimen, or need for its remounting. By contrast glycerine jelly permits specimens properly killed and cleared with lacto-phenol to retain their full roundness of shape, without the wrinkles of dehydration contraction. These detractions and one genuine merit are well known to technicians in parasitology. That the ova of pinworms suffer no great changes in dehydration is in this case less important than that the certain serious egg loss from the NIH swab has made us select glycerine jelly as mounting medium, notwithstanding its defects.

It is possible, on the other hand, to use a direct balsam mount of ova-laden scrapings, inverting the dry cellophane slip in the balsam immediately it is removed from the glass swab rod, flattening as before with a coverslip and sealing drop of balsam (method suggested by William Goode). However, the customary procedure of inverting the square in some watery fluid, its ova burden unknown, makes such direct balsam mounting less practical, negative preparations being pure waste of slide and coverslip. Using the balsam method, the contained embryos also tend to disintegrate, rather than to be killed and embalmed as is apparently done by the chemical preservatives of the glycerine jellies; the shell in such balsam mounting remains quite intact, is wholly typical in shape, and might suffice for teaching quick diagnosis of pinworm infections, rather than the details of internal ovum morphology. We have not thought such balsam mounting to be adequate for our particular purposes, and suggest rather that glycerine jelly is a satisfactory way to save from discard valuable teaching specimens of NIH pinworm ova scrapings.—David S. Ruhe, *Department of Bacteriology, Temple University School of Medicine, Philadelphia.*

EUPARYPHIUM MELIS (TREMATODA: ECHINOSTOMIDAE) FROM THE SNOWSHOE HARE

Trematodes as a group have rarely been recorded from North American hares and rabbits. Only three species, representing two genera, have been reported as occurring in leporines; and but one of these, *Hasstilesia tricolor*, has been reported from the snowshoe hare. The other two, *H. texensis* and *Fasciola hepatica*, have been recorded from the cottontail and the jack rabbit.

In over 600 examinations of snowshoe hares, *Lepus americanus phaeonotus*, we have found but one fluke—a single specimen of the echinostome, *Euparyphium melis*, in the stomach of one hare. This animal was collected by the Minnesota Wildlife Disease Investigation at Lake Alexander in Morrison County, Minnesota, on July 9, 1936.

The adult *E. melis* normally is a parasite of the intestines and stomachs of minks (*Mustela vison*). The metacercarial stage, as shown by Beaver, 1939, is passed in the tail muscles of tadpoles. It is possible that it may occur in fish too. Since hares occasionally eat dead frogs, tadpoles, and fish, they have the opportunity to become infected with *Euparyphium melis*. In our examinations of rabbits, we found in the cecum of one jack rabbit, *Lepus townsendii*, scales and bones of a sucker (*Catastomus*).

I wish to thank Dr. Paul C. Beaver and Mr. Seymour Feldman, who verified the identification of *E. melis*.—Arnold B. Erickson. *Paper No. 1811 Scientific Journal Series Minnesota Agricultural Experiment Station, St. Paul. In cooperation with the Minnesota Conservation Department, Division of Game and Fish.*

REVIEWS

PROTOZOOLOGY. By Richard R. Kudo. 689 pages, 291 illustrations. Charles C. Thomas, Springfield, Ill., 1939. 2nd edition. $6.50 postpaid.

In this second edition, Kudo's "Handbook of Protozoology," originally published in 1931, has been so greatly revised and enlarged as to constitute essentially a new book and to justify the change in title. The work opens with a brief introductory elementary chapter, followed by separate chapters on the ecology, general morphology, physiology, reproduction, and variation and heredity of the protozoa. Each of these chapters contains an excellent, though necessarily brief, discussion of its subject and includes the well established results of some recent experimental work. Together, these chapters occupy 170 pages and constitute a good introduction to a study of the biology of the protozoa. It is unfortunate that space did not permit a more thorough discussion, in the chapter on physiology, of recent precise work on the cultural requirements of protozoa. The work of Hall and his collaborators is mentioned but no references are given, while that of Lwoff is not even noted.

The bulk of the book consists of a taxonomic treatment of the phylum Protozoa. The general characters of each larger group are described and keys given to the subgroups within it down to the family. The genera in each family are briefly characterized and at least one species in each genus is listed and illustrated. The abundant and excellent illustrations, drawn in a uniform and very clear manner, are in themselves enough to make this "Protozoology" a valuable reference work wherever studies of either free-living or parasitic protozoa are being prosecuted. It is impossible to praise too highly the figures and their arrangement. Typographical errors are few and minor, except for Fig. 200, which is inverted.

The author has for the most part chosen to give only one side of problems still in a controversial stage. For example, in his discussion of the life cycle of *Babesia* only the views of Dennis, and not the opposing views of Reichenow, are given.

Appropriate references are listed at the end of each chapter, and the book closes with an adequate author and subject index.—W. TRAGER.

FLEAS OF EASTERN UNITED STATES. By Irving Fox. vii + 191 pages, 161 illustrations. Iowa State College Press, Ames. 1940. $3.00 postpaid.

"Fleas of Eastern United States," by Irving Fox of Iowa State College has been eagerly anticipated for several months. It is in no way disappointing. The author has limited his field to that part of the United States east of the 100th meridian, which passes through North and South Dakota, Nebraska, Kansas, Oklahoma, and Texas. Texas, however, he excludes entirely. This seemingly artificial boundary has long been recognized as approximating the eastern or western limit of many species of birds and mammals.

Treatment of each of the 55 species representing 33 genera and 5 families, accepted as occurring in the Eastern States, is divided into the following sections: (1) *Bibliography.* Arranged chronologically, including references to original description, occurrence in the area under consideration, synonyms and changes in nomenclature. (2) *Male.* Described in considerable detail. (3) *Female.* Description usually limited to modified abdominal segments. (4) *Records.* In which exact host, localities, dates, and names of collectors are included. These records are apparently limited to specimens actually examined or to selected records, while previously published records are not included unless the specimens were reviewed by Fox. There is no definite statement to this effect. Use of quotation marks around some host names in these sections seems inconsistent. (5) *Eastern Hosts* and (6) *Eastern Localities* summarize published and present records for hosts and localities by states only. (7) *Type Material.* Host, type locality, extent of type material and its deposition are given. A brief discussion of the species and its related forms is given in some cases.

Tunga penetrans (Linnaeus) is mentioned as possibly occurring in the Eastern States, but no authentic record is cited. Faust and Maxwell (1930, Arch. Dermat. and Syph. **22**: 94–97) record a most unusual infestation in a native of New Orleans where adult fleas were not found but living larvae were abundant in dermal abrasions. Records in the Eastern States of *Diamanus montanis* (Baker), *Peromyscopsylla selenis* (Rothschild) and *Ceratophyllus niger* Fox are questioned by Fox. *Ceratopsyllus crosbyi*, which was inadequately described by Baker, is the only species recorded for the area which remains unrecognizable. *Nearctopsylla hygini* (Rothschild) is reduced to a synonym of *Nearctopsylla genalis* (Baker), a name of uncertain status for many years. Several other synonyms are recognized. *Eptescopsylla*, a new genus of bat fleas, is described.

The study of a rather extensive series of critical specimens and species in the collection of the Rocky Mountain Laboratory leads me to question the following taxonomic changes: giving *Hoplopsyllus glacialis lynx* (Baker) full specific rank, reducing *Orchopeas caedens durus* (Jordan) to synonymy with *O. caedens caedens* (Jordan), and reassigning to *Megabothris* American species included in the genus *Monopsyllus* by Jordan.

Trichopsylla (= *Chaetopsylla* of some authors) is included in the family Dolichopsyllidae. Wagner (1930, Katalog der Palaearktischen Aphanipteren) considers this and several allied genera deserving of family status as the Vermipsyllidae. However, there is marked disagreement among taxonomists as to the number and scope of the families of Siphonaptera.

The *Synonymic Index* (3 pages) is extremely useful for quickly locating synonyms and obsolete combinations, which considerably outnumber the valid names. The *Host Index* is very complete, yet such extensive records (6 pages) as Fox has presented would justify including here some indication of normal parasites in contrast to accidental parasites resulting from habitat association or predation.

A paper by Harkema, "The Parasites of Some North Carolina Rodents," (1936, Ecol. Monogr. **6**: 151–232) should be added to the bibliography.

As the critical characters of fleas lend well to illustration and poorly to keys, verbal description or measurement, the extensive series of figures (166 figures, 31 plates) will be the most useful part of the book to those identifying specimens. Reference to plate pages is unfortunately omitted in the general index. The artistry in this section does not equal the composition and careful preparation of the text. Nevertheless, figures are clear and technically accurate, with very few exceptions.

Recent discoveries on the prevalence of sylvatic plague and flea-borne typhus in the United States have greatly stimulated interest in Siphonaptera and demand a better knowledge of our flea fauna. This text appears, therefore, at an opportune time for the parasitologist, entomologist, and public health worker, to all of whom it is recommended without reservation. It also deserves the attention of mammalogists and ornithologists. It is the only comprehensive taxonomic treatise on North American Siphonaptera since Baker's monographs of 1904 and 1905.—WILLIAM L. JELLISON.